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ABSCISSION UNDER THE INFLUENCE OF ETHYLENE IN CONNECTION WITH THE DISTRIBUTION OF PHYTOHORMONES OF THE BIOS GROUP

[Following is the translation of an article by Yu. V. Rakitin, Institute of Plant Physiology imeni K. A. Timiryazeva, AN USSR, Moscow, published in the Russian-language periodical Botanicheskiy Zhurnal (Botanical Journal) Vol 31, No 2, 1946, pages 11-16. It was submitted on 7 Jan 1946.]

Abscission, whether connected with the natural rhythm in the development of plants or caused by the disruption of normal conditions of vegetation, is a complex physiological problem.

In the majority of plants in the basal part of the foliate petiole there is a small transitional area, the tissue of which is very reduced; only the tracheal elements here usually turn out to be lignified, and the cells of parenchyma are strongly reduced and are characterized by a thicker cytoplasm. Shortly before abscission as a result of the division of cells of parenchyma in this area a secondary meristem is formed, the so-called separation layer, intersecting the entire petiole in a transverse direction. After this the cells of the separation layer are rounded and on the strength of dissolving of the middle blades are separated from each other, so that the leaf continues to be held back only by dead fibro-vascular bundles which finally are split and the leaf falls. In simpler and less frequent cases the formation of the separation layer takes place without cell division and amounts only to the separation of the latter by means of the dissolving of the middle blades as this takes place in Acer and Pyrus (Ims and Mak Daniels [1]). Thus the most typical formation of a separation layer is composed of the following two phases: the phase of embryonic division and the phase of mutual separation of the cells.

In spite of the fact that a long series of investigations has dealt with the study of abscission, the physiological "mechanism" of the phenomenon still remains a long way from being explained.

In our paper, published in 1939 (Rakitin and Yarkovaya [5]), we made an attempt to study this phenomenon in the light of its connection with bios, which is an activator of cell division. This bioactive factor was first discovered in yeast in 1901 by Professor Ido and his pupil Vilde at Luvenskiy University.

The capacity of bios to activate cell division is one of its most studied properties. This property, initially discovered on microorganisms, was subsequently noted in cells of higher plants (Kholodnyy [7], White [14]). In this respect there is particular interest in the papers by Gauthoret [12] and White [14, 15]. By cultivating (under aseptic conditions) fragments of the secondary cambium of willow on nutrient medium, Gauthoret detected that the intensity of cell division of the meristem is found in a specific dependence on the composition of the nutrient substrate. In the case of adding vitamin B_1, which is one of the main components of bios, to the substrate the intensity of cell division was noticeably increased. Analogous results were obtained in the experiments by White with cambium from the stem apice of tobacco under conditions where a bios-rich yeast extract was added to the substrate.

The connection between the meristematic activity of the tissues of higher plants and the content of group B phytohormones in them is also testified to by data from Dagys [8, 9, 11], Thimann and Bonner [13], and finally by the work of Yarkovaya [7a, 7b].

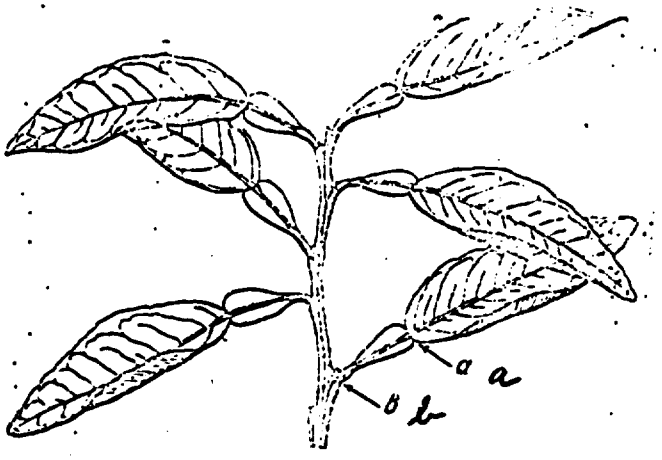

Figure 1. Sprout of cultivated lemon.

In tracing the distribution of bios in the leaves and stems of Pueraria (Pueraria Thunbergiana) and grape (Vitis vinifera), we arrived at the assumption that one of the main factors which causes the division of cells of the [illegible] layer, just as in other cases, [illegible] group (Nakitin and Yarkovaya [6]). [illegible] is the fact which we established that [illegible] content of bios in the [illegible] increased sharply, [illegible] increased [illegible] in the amount [illegible] of its influx [illegible] leaves before [illegible] and Gavrilov [,].

In the present report the results are cited from investigations relative to the falling off of leaves under the influence of ethylene in connection with the distribution of substances from the bios group. The study of this problem was carried out on potted plants of lemon, grapefruit, and wild lemon (Poncirus trifoliata); in all cases the procedure for setting up the experiments was the same.

Figure 2. Sprout of grapefruit.

Two similar sprouts (without removing them from the plant) were inclosed in different chambers (Bunsen flasks with a volume of 5 liters). The necessary hermetic conditions were achieved in the following manner. The sprouts which were placed in the flasks were fastened in the flasks on cork stoppers and were isolated from the outside atmosphere by plasticine. One of the chambers served as a control, and to the second we added (through a lateral tube) ethylene in a concentration of 1:5,000. The duration of each of the tests performed was 72 hours. The tests were carried out under hothouse conditions (at night - darkness, in the day - scattered light) at 20-25°C. The overall picture of changes observed turned out to be the same in all cases.

In 24 hours the leaves of the test sprouts became noticeably pallid, in another 24 hours signs of yellow coloring appeared in them, and finally after another 12-24 hours they began to fall off.

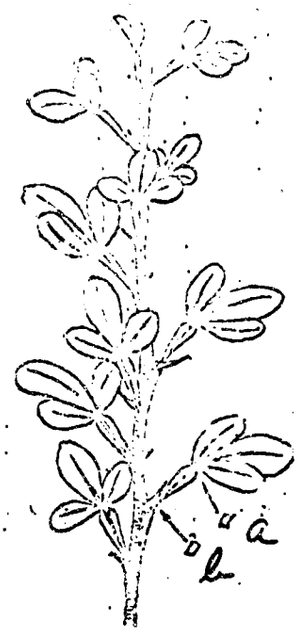

Figure 2. Sprout of wild lemon.

It was characteristic that at first only the leaf blades fell off, and the leaf stalks remained in place and fell off only after 2-3 days. As regards the leaves which were found on the shoots which served as the control, during the time of the tests they did not display any visible changes.

The noted sequence of falling off of parts of the leaves under the influence of ethylene indicates that the separation layer a, which is formed at the main leaf blade, is formed noticeably more rapidly than separation layer b which develops at the main stalk (Figures 1, 2, 3). Based on our observations the same order of formation of separation layers and the falling off of leaf blades and stalks can be observed in the investigated plants also during natural abscission.

In the tests for calculating the distribution of bios we used the yeast method described by Verner and Kling [1].

The samples of material necessary for the preparation of bios extracts were taken in 48 hours from the moment of charging the chamber with ethylene. The amount of material in each sample was 0.5 g (dry weight). The samples taken were cut into small fragments and after 25 ml of water was added to them they were subjected to 3 minutes of boiling. By means of decantation the extracts were poured into measuring flasks and water was added up to a volume of 50 ml. Into Erlenmeyer flasks containing 25 ml each of Gayduk medium, we added equal volumes of a suspension of bios depleted yeast (Saccharomyces cerevisiae) and equal volumes (2 ml) of bios extract. The subsequent cultivation of the yeasts was carried out at 25°C. Calculation of the growth of yeast was done in 48 hours from the time of inoculation. For calculating the yeast cells we used a Hellige counting chamber.

Judging by the results of the calculation the nature of the distribution of bios in all the investigated plants turned out to be monotypical. Table 1 is presented as an example of the regularity revealed.

The results of the test show that the content of bios increases in all the parts of plants treated with ethylene ; based on concentration of bios first place belongs to the leaf blades, in second

is the area of the separation layer a, in third - the leaf stalks, in fourth - the area of separation layer b, and finally in fifth - the bark of the stalk.

Table 1

Distribution of bios in the grapefruit plant

(a) Наименование проб	(b) Среднее число дрожжевых клеток (из 10 подсчетов) в поле зрения счетной камеры Фюллиге при объективе № 14 и окуляре Фюллиге.	
	(c) контроль	(d) этилен
(e) Листовая пластинка	62.3	226.6
(f) Область отделяющего слоя *a*	52.5	215.2
(g) Черенок	58.1	194.4
(h) Область отделяющего слоя *b*	58.2	205.1
(i) Кора стебля	55.3	82.5

Key: (a) Name of sample; (b) Average number of yeast cells (out of 10 calculations) in a field of vision of a Fellige counting chamber with objective No 14 and a Fellige eyepiece; (c) Control; (d) Ethylene; (e) Leaf blade; (f) Area of separation layer a; (g) Stalk; (h) Area of separation layer b; (i) Bark of stalk.

In examining these data it is not difficult to be convinced that the reason for this established distribution of bios is the circumstance that, developing in the greatest quantity in the leaf blades, it then reaches the stalk and moves in it in the direction of the stem. In connection with such an outflow pattern for bios it is also possible to explain why the separation layer a is formed somewhat earlier than separation layer b, and why at first the leaf blades fall off and only then the leaf stalks. This apparently takes place because in the area of separation layer a the concentration of bios which is necessary for the onset of cell division is reached somewhat earlier than in the area of separation layer b.

It was demonstrated in the experiments by Sukhorukov, Kling, and Klyachko [6] that in plant cells bios is found in two states: in solution and in a state of being bound with protein; during autolysis of plant material the bound bios passes into the solution. The conclusions of Sukhorukov and his associates found confirmation in the investigations by Lagys [9, 10]. Those data made it possible to expect that in leaves which are falling off the increase in the amount of free bios is the result of autolytic processes. For the purpose of establishing the feasibility of

such a route in the accumulation of free bios direct experiments on autolysis were undertaken. As test material the leaf blades of grapefruit and lemon were used.

For the different variants for the duration of autolysis (in each test there were 4 variants: 24, 48, 96, and 192 hours) we took 2 gram samples of material. The same value of weighed portion was selected for determining the initial content of bios. The autolytic mixture was made up of 2 g of ground plant material, 25 ml of water, and 2 ml of toluene. Autolysis was carried out at 25°C. Upon completion of the appropriate periods of autolysis the mixtures were boiled for the purpose of inactivating the enzymes and removing the toluene. After this the autolysates were subjected to autoclaving and then preserved in a sterile form up to the moment their activity was calculated. The same weighed batches (ground and mixed with water) which served for the calculation of free bios in the initial material were subjected to boiling and sterilization already in the beginning of the tests. When setting up the tests for the calculation of activity of bios in sterile extracts, the latter were brought up to 50 ml with water and then filtered. Subsequently the method for calculation of bios was the same as was described above. Based on the nature of the resulting data all the experiments conducted turned out to be completely analogous. The course of accumulation of free bios in the process of autolysis can be illustrated by Table 2, in which the results of one of the tests with grapefruit are presented.

Table 2

Release of bios during autolysis of the leaf blades of grapefruit

(a) Наименование пробы	Среднее число дрожжевых клеток (из 10 подсчетов) в поле зрения счетной камеры Hellige при объективе № 14 и окуляре Hellige (b)
Исходный материал (c)	30
Автолиз 24 часа (d)	85
Автолиз 48 часов (e)	159
Автолиз 96 часов (f)	240
Автолиз 192 часа (g)	420

Key: Name of sample; (b) Average number of yeast cells (out of 10 calculations) in a field of vision of a Hellige counting chamber with objective No 14 and a Hellige eyepiece; (c) Initial material; (d) Autolysis 24 hours; (e) Autolysis 48 hours; (f) Autolysis 96 hours; (g) Autolysis 192 hours.

It follows from Table 2 that the liberation of bios is found in a solid bond with the processes of autolytic decay; the more prolonged the autolysis the greater the amount of bound bios which is converted into a free state. If these data are compared with the well-known fact that processes of autolytic decay are taking place in the leaves which are falling off, then it will be clear that enrichment of the latter with bios is the result of autolysis.

Thus, it can be considered that during the falling off of leaves under the influence of ethylene the same changes are taking place as in the case of fall abscission. In both cases the falling off of leaves is preceded by a strengthening of processes of decay and the accumulation of free bios. Bios flows from the leaf blades into the leaf stalks and apparently causes cell division in them. The separation layers develop from these divisions.

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