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The dynamics of gibberellin-like and growth-inhibiting substances during seed development of Fraximus Excelsior L.*

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In the literature much attention has been given to the role of endogenous growth regulators, among others also gibberellin and growth inhibitors, in the phenomenon of dormancy and after-ripening of ash seeds (Villiers and Wareing 1960; Wareing and Villiers 1961; Villiers et al. 1963; Nikolaeva 1963; Szalai 1963; Villiers and Wareing 1965 a, b. As yet no systematical study has been made to examine the content of these substances in the course of seed development.

There exist some facts pointing towards the essential changes in the content of gibberellin-like substances during seed development of some grasses (Stoddart 1965; Reiowski 1964). Considerable changes in amount of these substances were also observed by Corcoran and Phinney (1962) in developing seed of *Echinocystis*, *Lupinus* and *Phaseolus*. Maheshwari and Johri (1965) investigated gibberellin-like substances during seed development of *Zephyranthes lancasteri*. In conclusion of the experiment it has been stated that these substances might have been closely related to the development of seed.

It seems possible that the changes in metabolism of some growth regulators in developing seed might also interfere with the further stages of their development. The analysis of fully matured seed does not give an exact idea about all potencial growth activities contained within the seed. Thus it seems very important to state the changes in the content of some growth regulating substances at the different stages of seed formation.

In the present paper the dynamics' of gibberellin-like substances at different periods of seed development of ash, *Fraxinus excelsior* L., was investigated. The quantitative changes in the content of these substances were studied from the moment of seed initiation till its full maturity.

Also the growth-inhibiting substances were examined in stratified and non stratified seeds and the biological characteristic of these compounds was performed.

METHODS

The content of gibberellin-like substances was examined at 11 different periods of seed formation, beginning three weeks after pollination: (I) 25.5., (II) 8.6., (III)

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22.6, (IV) 6.7., (V) 27.7., (VI) 17.8., (VII) 7.9., (VIII) 22.9., (IX) 13.10., (X) 3.11., and (XI) 24.11.1964.

Extraction and chromatography: For the first and second analysis 5 g of developing seeds were used (1- st analysis-seeds with covering structures, 2- nd analysis-naked seeds). In the further investigations 20 g of naked seeds were frozen, ground in a mortar and extracted with 70% acetone (twice during 24 hrs). The acetone of the combined extracts was evaporated in vacum at 35°C±2.

The remaining water extract, after acetone evaporation, was partitioned with ethyl acetate and phosphate buffer (pH = 7) by the method of Murakami (1959). The ethyl acetate fraction was evaporated and the residue was dissolved in a small volume of acetone and chromatographed on 20 cm long strips (paper: Whatman No 1-ascending technique; solvent system: n-butanol, acetic acid, water-ratio 19 : 1 : 6).

The detection of gibberellin-like substances on chromatograms was effected by spraying with 5% ethanolic sulphuric acid heating at 60° C (viewing under ultraviolet light), and by means of bioassay.

Bioassay: Chromatograms for bioassay were cut in 10 equal parts and each of them was eluated in 1 ml 2% saccharose during 6 hrs. The eluates were then applied to the oat's first leaf test as described by Michniewicz (1961). An estimate of the amount of gibberellin present was made by comparison of the growth of test plants with that of similar plants treated with known amounts of gibberellic acid (GA_3). Against this the concentration of the detected compounds was presented as an equivalent of GA_3 activity.

The activity of the present growth-inhibiting substances has been expressed in biological activity units. As one activity unit accepted was the growth-inhibition of 10% in relation to control plants.

In the diagram each of the given value equals the sum of activity units in the neighbouring sections of the inhibiting zones of the chromatogram.

Further characterization of the growth inhibiting substances, isolated by means of paper chromatography was also performed on the basis of their biological activity in relation to the *Avena* coleoptile straight growth test.

The influence of these inhibitors on the growth promoting effect of gibberellic acid in relation to the dwarf pea test was also investigated. In that case the eluates from the inhibiting zones of chromatograms were examined simultaneously with an appropriate concentration of gibberellic acid, chosen in accordance with the results of some preliminary experiments. The effectiveness of the combined solutions was bioassayed on peas, variety Kelvedon Wonder.

Preparation and chromatography of growth inhibitors. The growth inhibiting substances were estimated in dry stored ash seeds previously imbibed for 72 hours and in seeds after a low-temperature stratification. The extraction of the growth-inhibitors was carried out as described by Köves (1957). The ether-extractable acidiferous inhibiting substances were chromatographed with isopropanol-ammonia-water (10 : 1 : 1) solvent on Whatman No 1. The content of growth

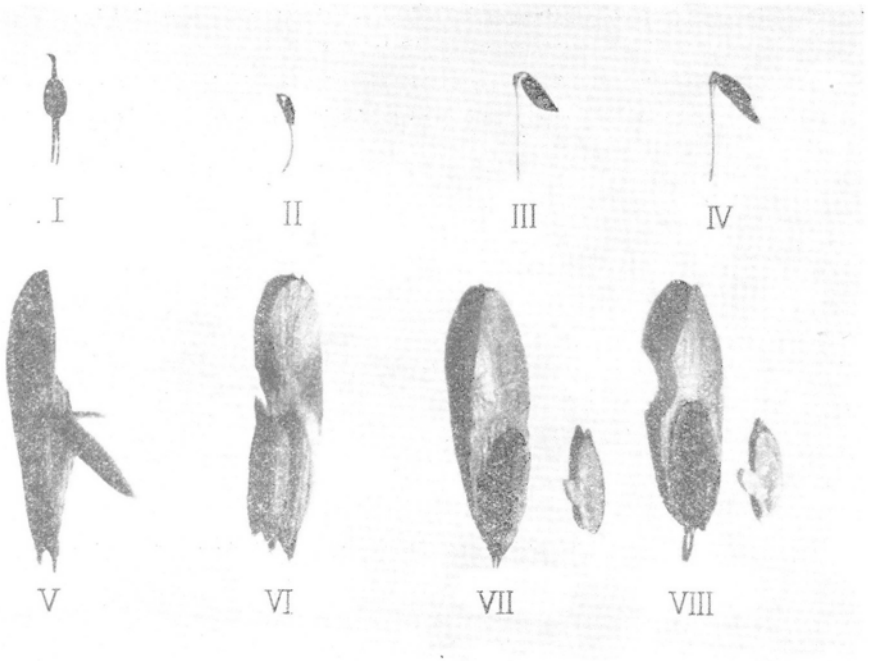

Fig. 1. The morphological state of the analyzed ash seeds.
Roman numbers- successive analyses as in Fig. 2. VIII — final phase of seed development

regulators in the eluates from the 2 cm strips was analyzed by applying the *Avena* coleoptile section test. The influence of the chromatographically separated inhibitors on gibberellin induced growth was also performed.

RESULTS

1. Analysis of gibberellin-like substances during seed development

The morphological state of the analyzed seed is demonstrated in Fig. 1.

The results of the successive analysis of gibberellin-like substances at the different stages of seed development are presented in Fig. 2. Generally, two growth promoting zones could be distinguished between *Rf* 0.8—1.0 (corresponding with the position of gibberellic acid in the control chromatogram) and between *Rf* 0.0—0.3.

The activity of the second zone was connected with the presence of certain compounds, or perhaps a group of compounds, which could not be exactly determined but were showing the typical reaction of gibberellin in relation to the applied biological test.

The gibberellin-like substances, localized in *Rf* 0.8—1.0, varied to a great extent at the different periods of seed development. A high level, of these substances, was shown in the early stages of seed formation (analysis III—V). In the further analysis a decrease in the content of these substances was found. The decreased amount

of the gibberellin-like substances in *Rf* 0.8—1.0 was accompanied by the appearance of the second active zone between *Rf* 0.0—0.3. A second period of intensified activity of gibberellin *Rf* 0.8—1.0 was shown by analysis made on 22.9.64 (VIII), after the seeds reached their full morphological maturity. In the final stages of

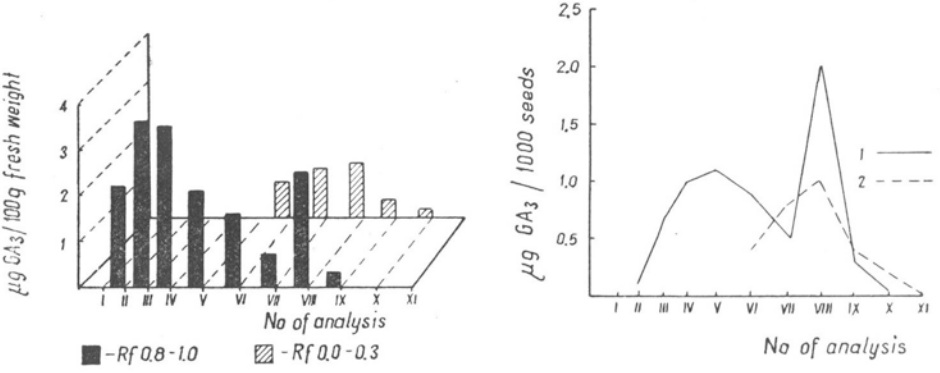

Fig. 2. Dynamics of gibberellin-like substances during seed development.
1 — *Rf* 0.8—1.0 *2* — *Rf* 0.0—0.3

seed development, the activity of both substances diminished markedly. In mature seeds no gibberellin-like substances were detectable.

In the developing seed also growth inhibiting substances could be observed (Fig. 3). The quite young seeds (I and II analysis) contained two inhibitors: one of a mild inhibitory action in *Rf* 0.1—0.2 and a second between *Rf* 0.3—0.5.

Further analyses did not show inhibiting zones in the chromatograms. In fully mature dry seeds only one inhibiting substance was present between *Rf* 0.3—0.5.

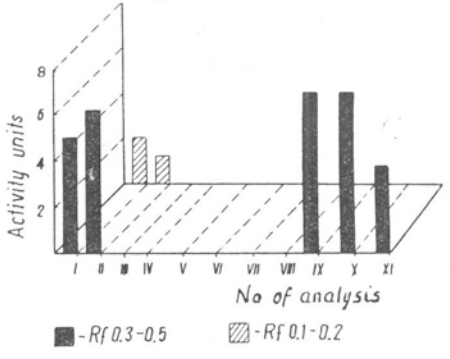

Fig. 3. The activity of growth-inhibiting substances during seed development (degree of inhibition in activity units).

The biological characteristic of the inhibitors, based on their activity in relation to different biological tests, has shown essential differences in the activity of these substances. The inhibitor present at *Rf* 0.1—0.2 have an inhibitory effect on the growth of *Avena* coleoptile sections. The same eluate, applied simultaneously with gibberellin, did not decrease its activity in relation to the dwarf pea test (Tabl. 1).

Table 1

The activity of inhibitors from developing ash seeds in relation to different biological test

Test	Avena straight growth tes			Dwarf pea test			
Treatment	Control	Eluates		Control		Eluates	
	Saccharose	E_1	E_2	—GA	+GA	E_1+GA	E_2+GA
Growth in mm	5.92	4.88	5.98	68.3	111.4	109.7	85.1
Growth in p.c.	100.0	82.4	101.0	100.0	163.1	160.6	124.5
L.S.D. P = 0.01	0.87			6.8			

E_1 — *Rf* 0.1—0.2 E_2 = *Rf* 0.3—0.5 GA = 0.1 μg/ml

Contrary to this, the inhibitor *Rf* 0.3—0.5, was inactive in relation to the *Avena* coleoptile test, but it has shown the ability to decrease the growth promoting effect of GA_3 (0.1 μg/ml) on the dwarf pea test.

2. Analysis of growth-inhibiting substances in stratified and non stratified seeds

The results of the bioassay of the chromatograms performed by the wheat coleoptile test are presented in Fig. 4. A strong inhibiting zone in the chromatograms was observed between *Rf* 0.6—0.9 in the stratified as well as in non stratified seeds.

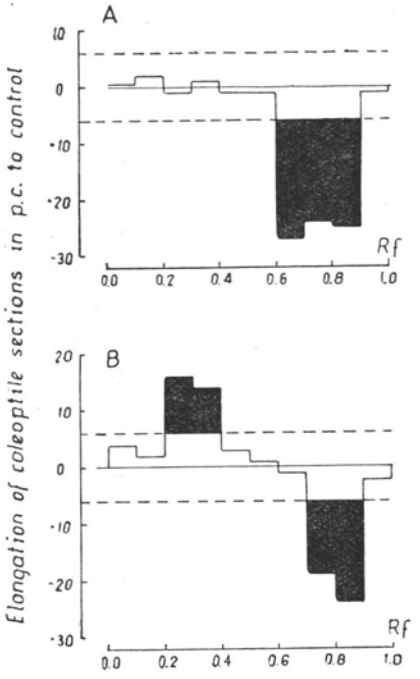

Fig. 4. Chromatographic analysis of growth-inhibiting substances in stratified and non stratified seeds (activity in relation to the *Avena* straight growth test).

A — non stratified seeds., *B*-stratified seeds. Black areas of diagrams represent significant differences.

The extracts from the seeds, after a low-temperature stratification, showed moreover the presence of a growth promotor (*Rf* 0.2—0.4), having the properties of auxin, but not identical with IAA.

The activity of the eluates applied simultaneously with GA_3 (0.005 μg/ml) was also investigated by the test of the first leaf of oat (Fig. 5). In the nonafter--ripened seeds two strong inhibitors of antigibberellic activity were observed. Extracts from seeds, after stratification, have shown the presence of only one substance of this type.

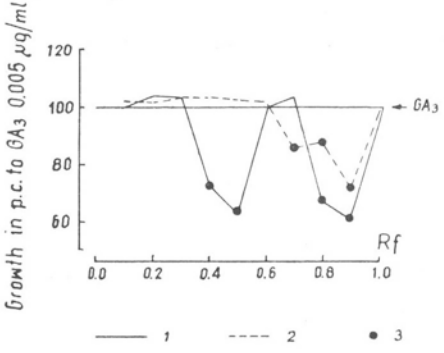

Fig. 5. The activity of growth inhibitors from stratified and non stratified seeds in relation to gibberellic acid (bioassayed by the test of the first leaf of oat).
1 — non stratified seeds *2* — stratified seeds *3* — significant differences

Similar results were also obtained by examining the influence of these inhibitors on the activity of GA_3 in relation to the dwarf pea test.

DISCUSSION

Great amounts of gibberellin were found in immature seeds of different plants (Radley 1958; Murakami 1961; Corcoran and Phinney 1962). The results of the present studies have confirmed those findings. The activity of gibberellin--like substances, showed a relatively high level in the early stages of seed development. The great activity of these compounds was probably connected with the process of a strong embryo formation which take place during that periode.

An intensified activity of these substances has been also noticed in seeds directly before they reached their full maturity. Mature ash seeds did not contain gibberellin--like substances but it might have been possible that they were present at such small amounts that their detection, by the method applied, was just impossible.

The second gibberellin-like substance localized in *Rf* 0.0—0.3 appeared after the seeds had achived their full morphological maturity and were able to germinate up to 50—64% when sown in green state.

The results of these experiments suggest that gibberellin may be an essential factor in the development of ash seeds. It might be also assumed that the development of these seeds is in fact, controlled by two seperate gibberellin-like substances showing different biological activity at the particular stages of seed development.

A considerable role during seed development may also play the observed growth inhibiting substances. This was also suggested by Rémy (1961). In the case of ash

seeds, two different kinds of growth inhibitors take part in the process of seed development — inhibitors of auxin, and gibberellin-inhibitors. In the dormant seeds some growth-inhibiting substances were also found, which showed the ability of decreasing the activity of exogenously applied gibberellin. In effect of the low-temperature stratification, the disappearance of some growth inhibitors showing antigibberellic properties was noticed (Fig. 5).

The activity of auxin-inhibitors remained at the same level in the stratified, as well as in the non stratified seeds.

It seems to be probable that biochemical processes, which occur during a low-temperature treatment of ash seeds, are leading to the disappearance of certain inhibitors of an antigibberellic nature which may be also involved in the system of factors limiting the normal growth and development of the embryo.

CONCLUSIONS

1. During the development of seeds of *Fraxinus excelsior* two different kinds of gibberellin-like substances were separated by means of paper chromatography. Their activity varied to a great extent at the different periods of seed development.
2. In fully matured seeds no gibberellin-like substances were detectable.
3. In the dormant seeds some growth-inhibiting substances were found, which showed the ability to decrease the activity of gibberellin.
4. Some inhibitors showing an antigibberellic character disapeared in seeds during the process of stratification.

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Dynamika substancji giberelinopodobnych i inhibitorów wzrostu w rozwoju nasion jesionu (Fraxinus excelsior l.).

Streszczenie

Oznaczano zmiany w zawartości substancji giberelinopodobnych i inhibitorów wzrostu w różnych fazach rozwoju nasion od momentu ich zawiązania do pełnej dojrzałości.

Zbadano także inhibitory nasion stratyfikowanych i niestratyfikowanych. Analizę substancji giberelinopodobnych podczas formowania nasion przeprowadzono w 11 okresach: (I) 25.5,(3 tygodnie po zapyleniu), (II) 8.6., (III) 22.6., (IV) 6.7., (V) 27.7., (VI) 17.8., (VII) 7.9., (VIII) 22.9., (IX) 13.10., (X) 3.11., i (XI) 24.11.1964.

Badany materiał roślinny ekstrahowano w 70% acetonie. Pozostałość wodną po odparowaniu acetonu frakcjonowano według metody Murakami'ego (1959) i badano chromatograficznie. Chromatografię przeprowadzano techniką wstępującą na bibule Whatman No 1. Jako układ rozwijający stosowano n-butanol:kwas octowy : woda (19:1:6). Aktywność eluatów z poszczególnych odcinków chromatogramu badano metodą biologiczną stosując test pierwszego liścia owsa. Identyfikację badanych substancji przeprowadzono na podstawie reakcji barwnej z H_2SO_4 w świetle U.V. Aktywność eluatów zawierających inhibitory badano także owsianym testem cylindrycznym i testem karłowatego grochu. Oznaczano również wpływ wyodrębnionych chromatograficznie substancji hamujących z nasion stratyfikowanych i niestratyfikowanych na efekt wzrostowy wzbudzany działaniem gibereliny.

Wyniki niniejszych badań pozwoliły na wysunięcie następujących wniosków:

1. W procesie rozwoju nasion jesionu uczestniczą przynajmniej dwa różne związki typu gibereliny, których zawartość ulega bardzo istotnym zmianom w poszczególnych etapach rozwoju nasion.

2. Nasiona w pełni dojrzałe wykazywały obecność inhibitora, nie zawierały natomiast substancji typu gibereliny.

3. W wyniku stratyfikacji nasion następowało zanikanie pewnych inhibitorów o własnościach antygibereliny, a pojawiał się stymulator, którego nie znajdowano w nasionach nie stratyfikowanych.

4. Wysunięto przypuszczenie, że zanikający w procesie stratyfikacji inhibitor może stanowić jeden z czynników ograniczających normalny wzrost i rozwój embrionu.

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