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Changes in the endogenous growth substances and the regulation of dormancy in the seeds of mazzard (*Prunus avium* L.)[1]

I. INTRODUCTION

Seeds of many species of plants, particularily from the region of the temperate climate, enter a state of rest during maturation. The dormant condition is according to Vegis (1964) genetically fixed in the plant

[1] Praca została wykonana w Zakładzie Dendrologii i Arboretum Kórnickim Polskiej Akademii Nauk w Kórniku. Autor przedstawił pracę jako rozprawę dok-

as an adaptation, and it can be caused by various factors; mechanical or chemical. Such fully viable seeds do not germinate in spite of being provided by appropriate conditions for it. For germination they require specified conditions that would act for a certain time — this refers particularity to temperature, humidity of air and sometimes also illumination.

The breaking of dormancy both in natural conditions as well as in the nursery practice takes place during the stratification treatment. The optimal thermal conditions for stratification of the seeds of mazzard (*Prunus avium* L.) have been experimentally identified by Suszka in 1962. He has shown that in the conditions of warm-followed-by-cold stratification (2 weeks at 20°C and 30 weeks at 3°C) the seeds of mazzard germinate to a much greater percent than during stratification at a constant low temperature (3° - 5°C) that was commonly recommended (Woody Plant Seed Manual 1948). Warm-followed-by-cold stratification has also proved best for the seeds of *Prunus cerasifera, P. mahaleb, P. serotina* and *P. armeniaca* (Suszka, 1967). The results of Suszka have been later confirmed for the seeds of mazzard by Joley (1967).

Physiological studies on the seeds of mazzard as concerns their dormancy have been conducted by Suszka (1962, 1966 and 1967) and Krawiarz (1970) who have worked on seeds stratified by the warm-followed-by-cold stratification and by other authors who have employed only the cold stratification, namely Pollock and Olney (1959), Olney and Pollock (1960), Pillay (1962), Pillay, Brase and Edgerton (1965), Pillay and Edgerton (1965), Proctor and Dennis (1968).

Pollock and Olney have studied the respiration of mazzard seeds. Pillay, Brase and Edgerton have studied auxins and Proctor and Dennis gibberellins in these seeds.

The studies conducted so far on growth substances in dormant seeds are fragmentary and sometimes even contradictory. In this respect the seeds of mazzard stratified by the warm-followed-by-cold method have not been studied.

The main purpose that the author has set himself was to find out what changes take place in the content of growth substances (auxins, gibberellins and inhibitors) and phenolics during the stratification and to see what relation they have to dormancy. Physiological studies were aimed at finding relations between the content of growth substances and the actual physiological state in relation to dormancy of the seeds.

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torską w Instytucie Biologii Stosowanej Akademii Rolniczej w Poznaniu. Promotor: prof. dr Mirosław Tomaszewski, kierownik pracowni fizjologicznej Zakładu Dendrologii i Arboretum Kórnickiego PAN w Kórniku.

II. MATERIALS AND METHODS

THE SEED MATERIAL

Seeds of mazzard have been obtained for the experiment in the years 1969 and 1970 from trees growing in the Kórnik Arboretum. Use was also made of seeds bought from CNOS (State Agency for the Distribution of Garden and Nursery Seeds) in 1969 in Poznań, the origin of these seeds however is not known.

The stones of mazzard prior to their use in the experiments have been stored in closed glass containers at a temperature of +1°C. In these conditions the seeds of mazzard maintain viability for at least 5 years at an unchanged level (S u s z k a, 1970).

The stones were stratified by the step-wise method, optimal for the seeds of mazzard (S u s z k a, 1962), using large lots and at appropriate times taking samples for the studies. In a parallel experiment the course of stone cracking and seed germination has been followed.

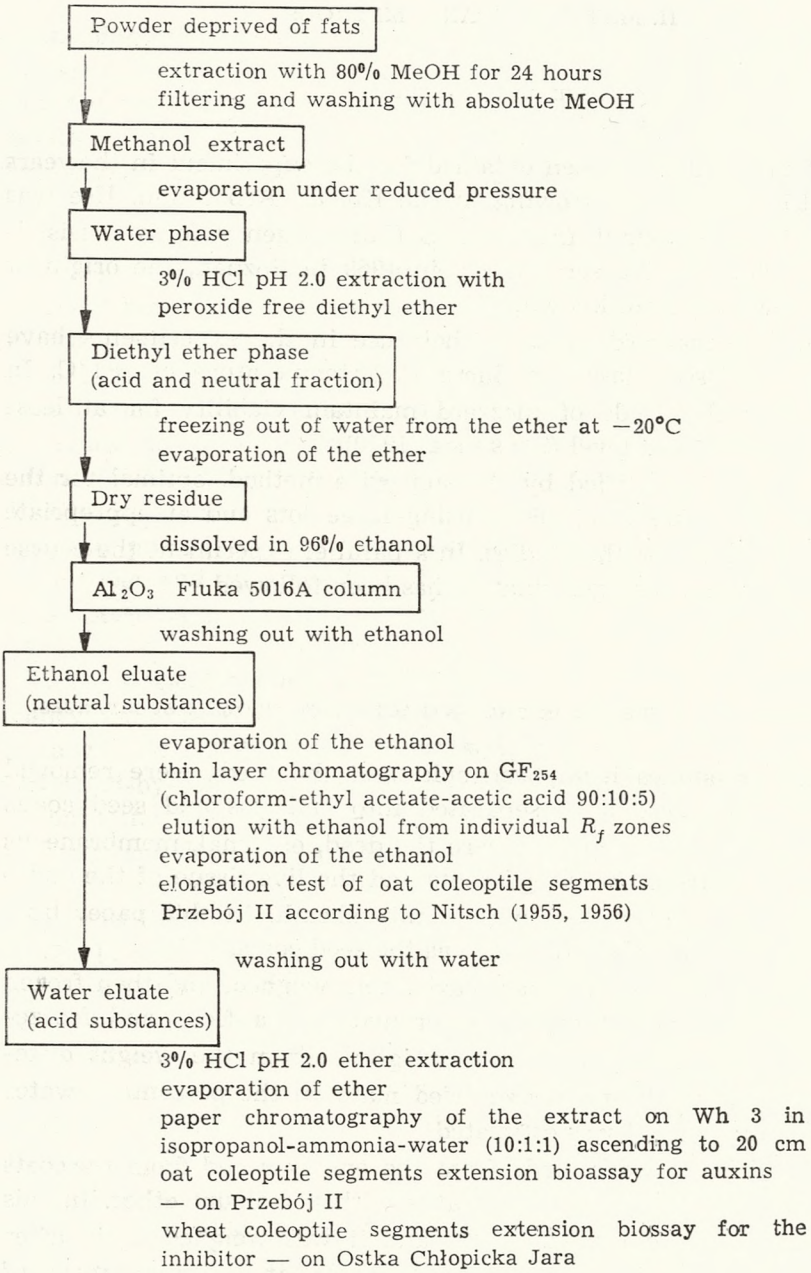
PREPARATION OF THE SEEDS FOR THE EXTRACTION OF GROWTH SUBSTANCES

The stratified stones have been split and the seeds were removed from them. These were later separated into embryos and seed coats. The seed coat is meant to include here the dead, external, membraneous cover that formed from the integuments and the live tissue of the endosperm that covers the embryo with a thin sheath. In this paper both these covers will be jointly referred to as the seed coats.

The embryos and the seed coats have been weighed, and then frozen in dry ice. The frozen material was ground into a flour and freeze-dried. The freeze-dried powder was weighed. From the weight difference between the fresh and freeze-dried material the percentage water content in the dry weight was estimated.

The freeze-dried powder made from the embryos and from the coats has been extracted in a Soxlet apparatus with petroleum ether. In this way the powder was deprived of fats and it was weighed again after which is was stored in a vacuum desiccator at a temperature of +1°C. In the stored material no changes have been observed concerning the activity of growth substances even after a year of storage. Extraction of auxins and of the inhibitor, and their separation on a column with aluminum oxide Al_2O_3 Fluka 5016A.

The auxins and the inhibitor have been extracted by the method of L a r s e n 1955 according to the following procedure:



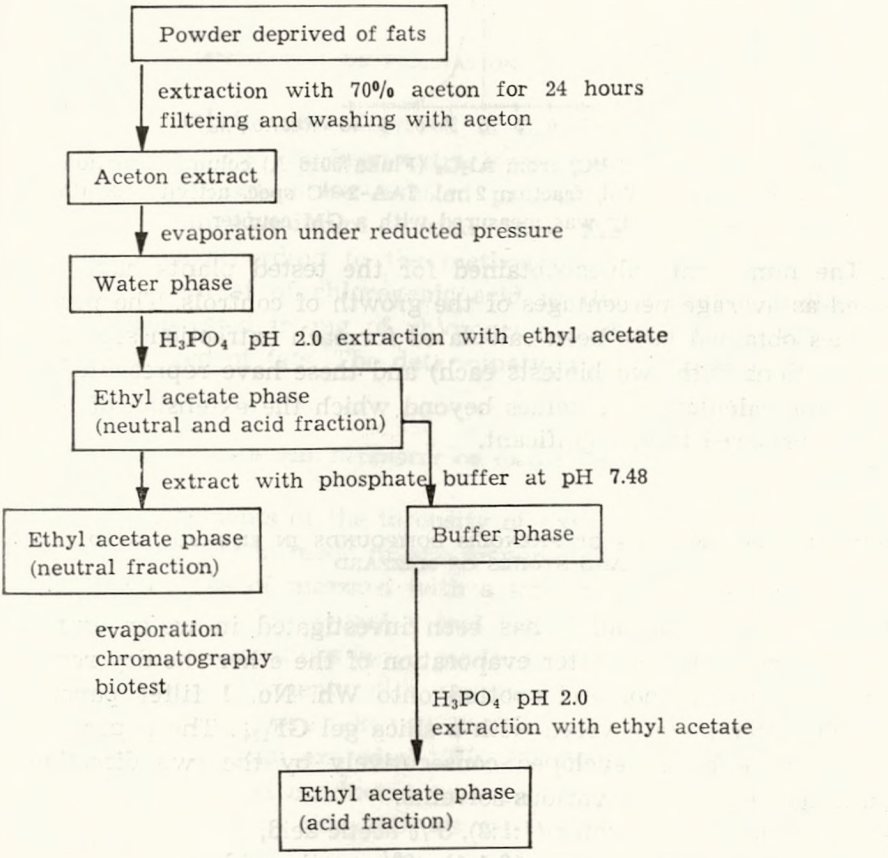
A trial was made to separate IAA from ABA using aluminium oxide column (Fluka 5016 A), according to the method of Linser (1954). The behaviour of indole-3-acetic acid on the column has been followed using the labelled IAA-2-^{14}C. The elution profile is presented in Fig. 1. However, when ABA was applied to the column it was found in the same fraction as IAA.

AVENA CURVATURE TEST

The presence of IAA in the embryos of the studied seeds of mazzard has been demonstrated by the oat coleoptile curvature test on Przebój II according to the method of Boysen-Jensen with Larsen's (1955) modification.

EXTRACTION OF GIBBERELLINS FROM EMBRYOS AND SEED COATS

The gibberellins were extracted following the procedure according to the method of Murakami (1959).



In the studies on the content of growth substances care was taken to maintain identical conditions during the initial preparation of the material and during the extraction cycle. Together with the studied material standard preparations of gibberellin GA_3, auxin IAA and abscisic acid ABA have been used and as a result it is possible to identify these substances and to calculate their quantities in the studied ma-

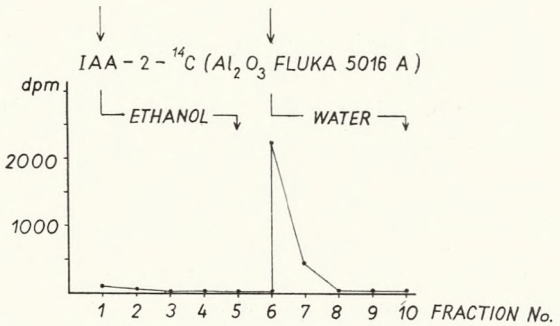


Fig. 1. Elution pattern (IAA-2-^{14}C) from Al_2O_3 (Fluka 5016 A) column. Fraction 1 - 5 in ethanol, 6 - 10 in water. Vol. fraction 2 ml. IAA-2-^{14}C spec. activity 9.3 μC/mM. Radioactivity was measured with a GM counter

terial. The numerical values obtained for the tested plants have been expressed as average percentages of the growth of controls. The percentage values obtained have been calculated for each extraction separately (two extractions with two biotests each) and these have represented the basis for the calculation of values beyond which the extension of histograms is considered to be significant.

CHROMATOGRAPHIC ANALYSIS OF PHENOLIS COMPOUNDS IN EMBRYOS, SEED COATS AND STONES OF MAZZARD

The presence of phenolics has been investigated in water extracts and in the ether fraction. After evaporation of the ether the dry residue was dissolved in ethanol and spotted onto Wh. No. 1 filter paper or onto a thin layer plate covered with a silica gel GF_{254}. The paper chromatograms have been developed consecutively by the two directional ascending technique using various solvents:

n-butanol-acetic acid-water (4:1:2), 3% acetic acid,
isopropanol-ammonia-water(10:1:1), 3% acetic acid,
benzene-acetic acid-water (4:1:2 organic phase), 3% acetic acid.

The thin layer chromatograms have been developed one way in the following solvents:

chloroform-ethyl acetate-acetic acid (90:10:5),
chloroform-ethyl acetate- acetic acid (60:40:5),
benzene-acetic acid-water (4:1:2 organic phase).

The dried chromatograms have been observed under UV light (254 and 365 nm) and the fluorescing or absorbing spots have been marked off. Further observations of these chromatograms have been conducted in ammonium fumes, then after spraing with 5% Na_2CO_3 and later after spraying with 5% NaOH. Finally the chromatograms have been sprayed with diazotized sulphanilic acid. The flavonoids have been differentiated by spraying with 1% $AlCl_3$ in ethanol (S e i k e l, 1964). The aldehydes have been developed with 2,4-dinitrophenylhydrazine. If in all the studied variants of the solvents and techniques the R_F values, fluorescence and colour reactions were identical with the standards (chemically pure substances) than it was assumed that the given substance was identified.

QUANTITATIVE DETERMINATION OF CHLOROGENIC ACID

The material (powder deprived of fats made from the embryos or from the seed coats) has been extracted with 80% methanol. The methanol was evaporated under reduced pressure. The water fraction was spotted onto an aluminium column Al_2O_3 Fluka 5016 A. The further procedure was according to the method of Z u c k e r and A h r e n s (1958). The content of chlorogenic acid in the material studied by us has been expressed in mg of chlorogenic acid per 100 g of the dried powder deprived of fats. The determinations were made in 3 replicates.

MEASUREMENTS OF THE INTENSITY OF OXYGEN ABSORPTION BY EMBRYOS

The measurements of the intensity of oxygen absorption by embryos of mazzard have been made by the manometric method. Into a Warburg vessel 10 embryos of mazzard with a known weight and volume have been placed. Into the central vessel 0.2 ml of 10% KOH have been supplied. The observations were made at a temperature of 20°C in 2 replicates. The experiments with oxygen absorption have been repeated twice. The readings were taken at 15 min. intervals. Deviations between the replicates have not exceeded 10%. All the experiments with oxygen absorption have been conducted in semisterile conditions. The amount of oxygen absorbed by the embryos was expressed in µl of oxygen per 100 mg of dry weight.

DETERMINATION OF PROTEIN CONTENT IN THE EMBRYOS

Determinations of protein content have been made by the Kjeldahl method through analyzing nitrogen. The nitrogen was determined in samples of the freeze dried material, deprived of fats, from which the

soluble nitrogen fraction has been removed with 70% acetone. The amount of protein in the embryos was expressed as the percentage of protein nitrogen in the defatted, powdered embryos extracted with 70% acetone. The determinations were made in three replicates.

III. RESULTS

1. PHYSIOLOGICAL CHARACTERISTIC OF STRATIFIED SEEDS

a) The breaking of seed dormancy during stratification

The course of stone cracking and seed germination during warm--followed-by-cold stratification of mazzard seeds is presented in Fig. 2. The cracking of stones begins after 9 weeks of stratification in the cold

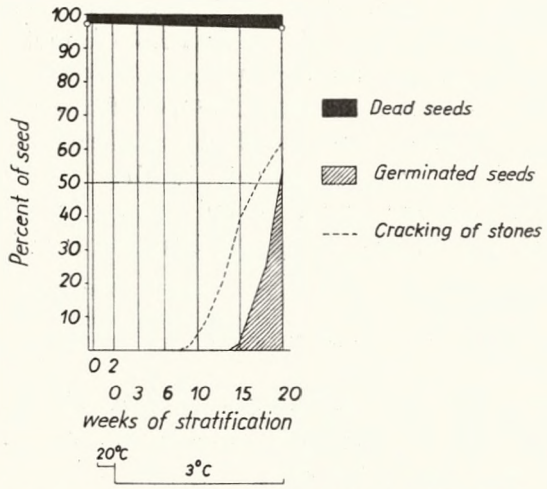


Fig. 2. The course of stone cracking and seed germination from mazzard (*Prunus avium* L.) during warm-followed-by cold (2 weeks 20°C and then 3°C) stratification

conditions. After 15 weeks of stratification at 3°C 40% of the stones are cracked. Germination of the seeds studied by us begins after 15 weeks. In the 20th week almost half of the stratified seeds were germinated. Such a pattern of dormancy breaking in the seeds of mazzard during stratification is quite regular.

b) Changes of water content in stratified seeds

Changes of water content in the embryos and in the seed coats during stratification are presented in Fig. 3. The water uptake by embryos and by the seed coats has two phases. In the first phase (imbibition) there

is a reconstitution of the moisture conditions that existed in the fresh seeds. Until the stones crack the moisture level is maintained at one level. Only after the seeds are transferred from the warm stratification at 20°C to the cool conditions (3°C) that a drop in the water content of the embryos and of the seed coats is observable. In cracked stones as well as in germinated seeds a much higher moisture level was found,

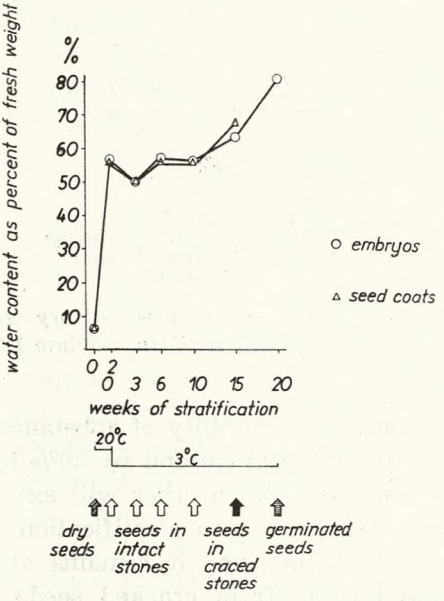


Fig. 3. Change in the water content (as percentage of fresh weight) in the seeds of mazzard (*Prunus avium* L.) during stratification

both in the embryos and in the seed coats, this being the result of water uptake during the second phase. It appears that the moment of stone cracking is associated with a considerable increase in the water content of the seeds coats. The ability to absorb such quantities of water by the seed coats (12.2%) is related to the physiological state of the seeds and is indicative that dormancy has been broken in them. This is also associated with the role seed coats play in the physiology of stone seeds. They participate in the process of stone bursting.

c) Changes in the content of fatty substances during stratification of seeds

In the studied seeds of mazzard the fats were extracted with petroleum ether. The results are presented in Fig. 4. The embryos of mazzard contain 35.0% of fat. The amount of fats in the embryos during stratification does not undergo changes. In the embryos from cracked stones the amount of the petroleum ether soluble fraction slightly increases. Germinated embryos contain 19.0% of fats less than embryos from the

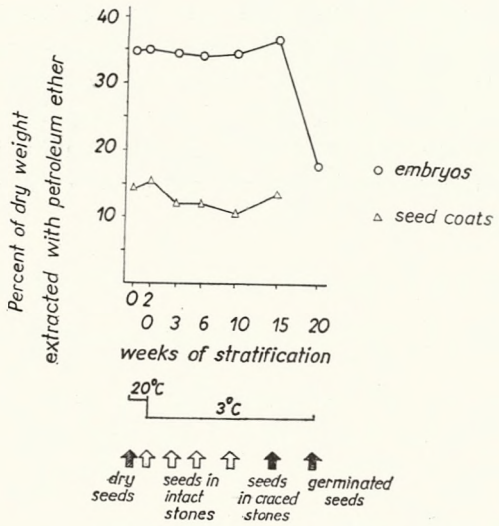


Fig. 4. Change in the fat content (as percentage of dry weight extracted by petroleum ether) in the seeds of mazzard (*Prunus avium* L.) during stratification

stage of cracked stones. The solubility of substances form these embryos increases by 12.8% in 70% acetone and by 20% in 80% methanol.

From the seed coats petroleum ether will extract about 15% of dry weight. After the period of warm stratification this quantity slightly declined, but throughout the time it remains at a more or less even level. From the seed coats from cracked seeds petroleum ether will extract only slightly more of fat. Again there are no major differences following extraction with acetone and methanol.

Table 1

Protein content in stratified embryos of mazzard (*Prunus avium* L.)

Stage of the embryos in the stratification treatment	Percentage of protein nitrogen on dry weight basis
Unstratified embryos	4.77
Stratified 2 weeks at 20°C	4.42
Stratified 2 weeks at 20°C and then 3 weeks at 3°C	4.30
Stratified 2 weeks at 20°C and then 6 weeks at 3°C	4.25
Stratified 2 weeks at 20°C and then 10 weeks at 3°C	3.68
Stratified 2 weeks at 20°C and then 15 weeks at 3°C (cracked stones)	3.41
Stratified 2 weeks at 20°C and then 20 weeks at 3°C (germinated)	3.05

d) Protein content in stratified seeds

Embryos of mazzard contain about 5,5% of protein nitrogen (about 10% of the insoluble fraction). Results of the determinations are presented in Table 1.

In the stratified embryos the content of proteins slightly declines, however it was only in the germinated embryos that a considerable drop in the protein fraction was observed. At the same time a considerable increase of solubility in 70% acetone and in 80% methanol was observed. In 70% acetone 44.0% of the dry, defatted embryo powder is dissolved while in 80% methanol 43.9%. The increase from the situation at the stage of cracked stones is respectively by 12.8% and 20.0%.

e) Oxygen absorption by the embryos

Studies on the absorption of oxygen by the seeds were aimed at deciding to what degree the stone shell (endocarp) hinders the uptake of oxygen by the embryo. Results of the measurements are presented in Fig. 5. As can be judged from the data presented differences in the ability to absorb oxygen by embryos with and without the seed coats and stone shells are considerable. Embryos without the seed coats absorb much more oxygen than when inside seed coats and less still in intact stones.

Results of the experiments with oxygen absorption by embryos are presented in Fig. 6. Embryos from freshly collected seeds absorb during 150 minutes about 50 µl 0_2 calculated per 100 mg of dry weight. During the swelling of embryos while in stratification at 20°C the oxygen absorption level is comparable to that in fresh seeds. This level is attained by embryos already after 48 hours of stratification. During the stratifi-

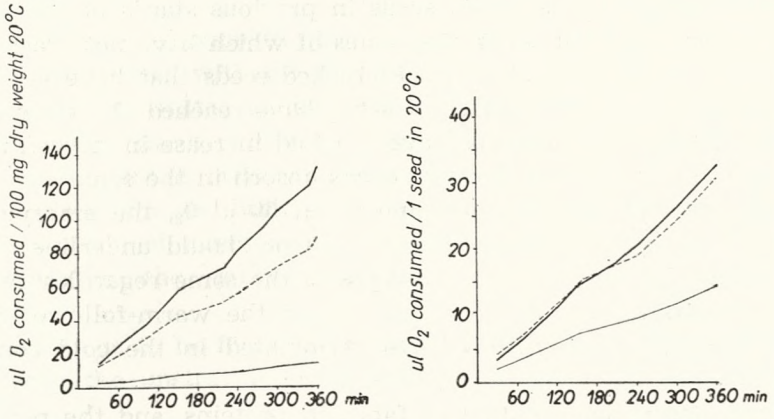


Fig. 5. Oxygen consumption by mazzard (*Prunus avium* L.) seeds

———— embryos, - - - - - - - - seeds, ———— seeds in intact stones

cation the amount of absorbed oxygen is maintained at a more or less even level established at the beginning. In the uncracked seeds, from which embryos were also taken for observation, the absorption of oxygen continues to be maintained at the same level. A very considerable increase in oxygen absorption by the embryos is observed only after the

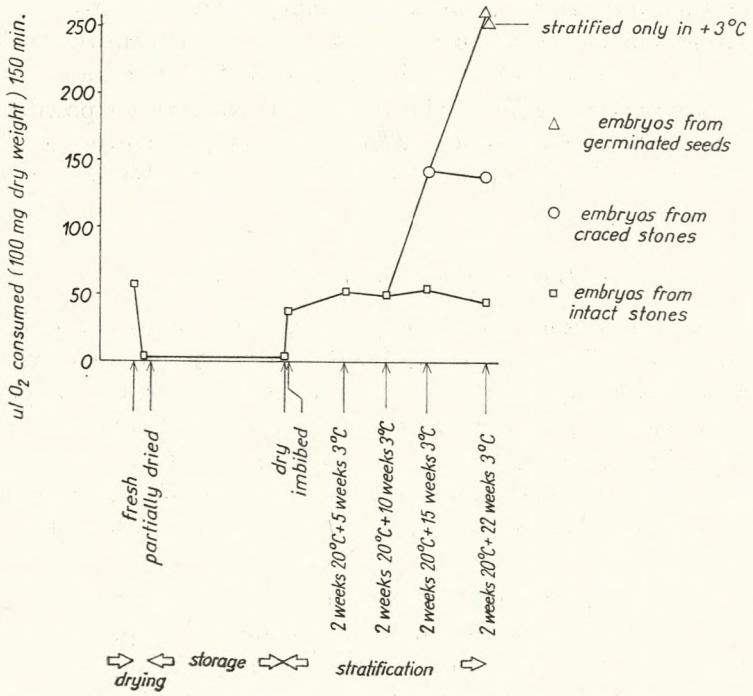


Fig. 6. Oxygen uptake by mazzard (*Prunus avium* L.) embryos

stage of stone cracking was reached. The embryos absorb then 3 times as much oxygen as those from seeds in previous stages of the stratification, or from parallel seeds the stones of which have not cracked yet. This level is maintained also in the cracked seeds that have not germinated even though the majority of seeds have reached the stage of germination. Germinated embryos have a 5 fold increase in oxygen absorption. While embryos from dormant seeds absorb in the same experimental conditions and in the same time about 50 µl 0_2, the embryos from germinated seeds absorb about 250 µl 0_2. One should underline that the absorption of oxygen by these embryos is the same regardless whether they have germinated in the conditions of the warm-followed-by-cold stratification or whether they have germinated in the cold conditions only.

Oxygen absorption, content of fats and proteins, and the percentage content of the acetone and methanol soluble fraction all appear to be interconnected and related to the stage of seed dormancy.

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2. CONTENT OF AUXINS IN STRATIFIED SEEDS

a) Activity of auxins in the mazzard embryos

The extraction of auxins from the embryos has been conducted by the described method. The dry residue following evaporation of the water extract from the Al_2O_3 Fluka 5016 A column has been picked up in 1.5% fluid agar, from which cubes were made. The results obtained in the coleoptile curvature test are presented in Table 2.

Table 2

Auxin activity in the mazzard embryos as measured by the *Avena* curvature test

Stage of development of the studied embryos	Weight of the analysed sample in g (2 ml 1.5% agar)	Degree of curvature
Unstratified embryos	2·4916	29.2
Embryos stratified 2 weeks at 20°C	2·6916	9.8
Embryos stratified 2 weeks at 20° and then 3 weeks at 3°C	2.4220	11.5
Embryos stratified 2 weeks at 20°C and then 6 weeks at 3°C	2.5932	13.2
Embryos stratified 2 weeks at 20°C and then 10 weeks at 3°C	1.8144	9.4
Embryos stratified 2 weeks at 20°C and then 20 weeks at 3°C (germinated)	0.5	0.0
	1.0	7.5
	2.0	13.0
Standard IAA solutions	25 µg/l	9.9
	50 µg/l	18.5
	100 µg/l	25.9

The activity of auxins in the extracts from mazzard embryos has been also determined with the help of the oat coleoptile extension test. The results are presented in the form of histograms in Fig. 7. From the presented histograms it can be seen that the zone corresponding to IAA on the chromatograms demonstrates an increasing activity as stratification progresses. On the basis of the activity of control IAA solutions the concentration of auxin in the studied samples was calculated. The auxin content in the embryos calculated in this way is presented in Table 3.

Most auxin is to be found in embryos from cracked stones. In comparison with the unstratified embryos they contain 10 times as much auxin.

The substance in the R_F zone 0.2 - 0.5 (in isopropanol-ammonia-water 10 : 1 : 1) is probably IAA. This is indicated by the biological tests and

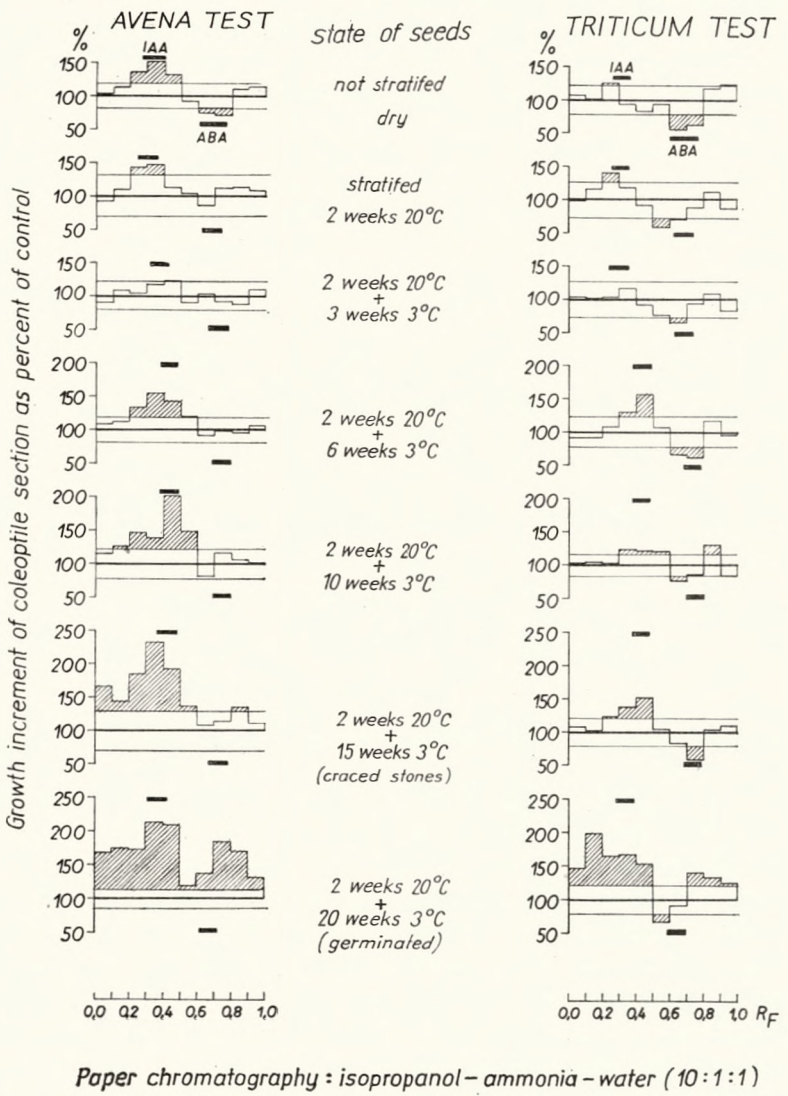

Fig. 7. Histograms of the bioactivity of the acidic ether fraction from mazzard (*Prunus avium* L.) embryos after separation on Al_2O_3 column (water effluent)
The extracts have been chromatographed on Whatmann No. 3 filter paper, isopropanol-ammonia-water (10 : 1 : 1). Segments of chromatograms were bioassayed by wheat coleoptile elongation test (growth inhibitors) and by the oat coleoptile elongation bioassay (growth promoters). Each histogram represents the activity of extracts from 60 embryos. Horizontal lines mark off the least significant difference from controls (100%) at a confidence level of $p=0.95$

Table 3

Auxin content in the embryos in IAA equivalents

Stage of development of the studied embryos	IAA equivalents in µg/1 kg sm	
	oat coleoptile extension test	*Avena* curvature test
Unstratified embryos	6.61	9.55
Embryos stratified 2 weeks at 20°C	4.94	7.05
Embryos stratified 2 weeks at 20°C and then 3 weeks at 3°C	3.1	4.6
Embryos stratified 2 weeks at 20°C and then 6 weeks at 3°C	8.5	12.0
Embryos stratified 2 weeks at 20°C and then 10 weeks at 3°C	19.8	27.9
Embryos stratified 2 weeks at 20°C and then 15 weeks at 3°C (from cracked stones) R_F 0.1	4.7	
R_F 0.2 - 0.6	60.5	
R_F 0.9	1.95	
Embryos stratified 2 weeks at 20°C and then 20 weeks at 3°C (germinated) R_F 0.1	3.69	
R_F 0.2 - 0.5	31.62	34.0
R_F 0.9	13.3	

by chromatography. The substance is active in the Avena curvature test, which is highly specific for auxin. The other two substances active in the biotests it was not possible to identify at the moment.

b) Activity of auxins in the seed coats

Studies on the content of substances having an auxin type activity have been conducted also in the seed coats. The results are presented as histograms in Fig. 8. In the seed coats from dormant seeds a substance active on the oat coleoptile segments growth has been found. It occupies a zone on the chromatograms following development in isopropanol-ammonia-water (10 : 1 : 1) with an R_F of 0.3 - 0.6, similarily as the substance from the embryos. It is probably IAA. Activity in the dormant phase is small and falls to zero after the period of warm stratification. During stratification in cold conditions the activity of this auxin increases at first, and a maximum of activity was observed in the seed coats of seeds that have been stratified in the cold conditions of 3°C for 6 weeks. From that moment a drop in activity in that zone was observed, so that at the next analysed stage no auxin activity was detected in the seed coats.

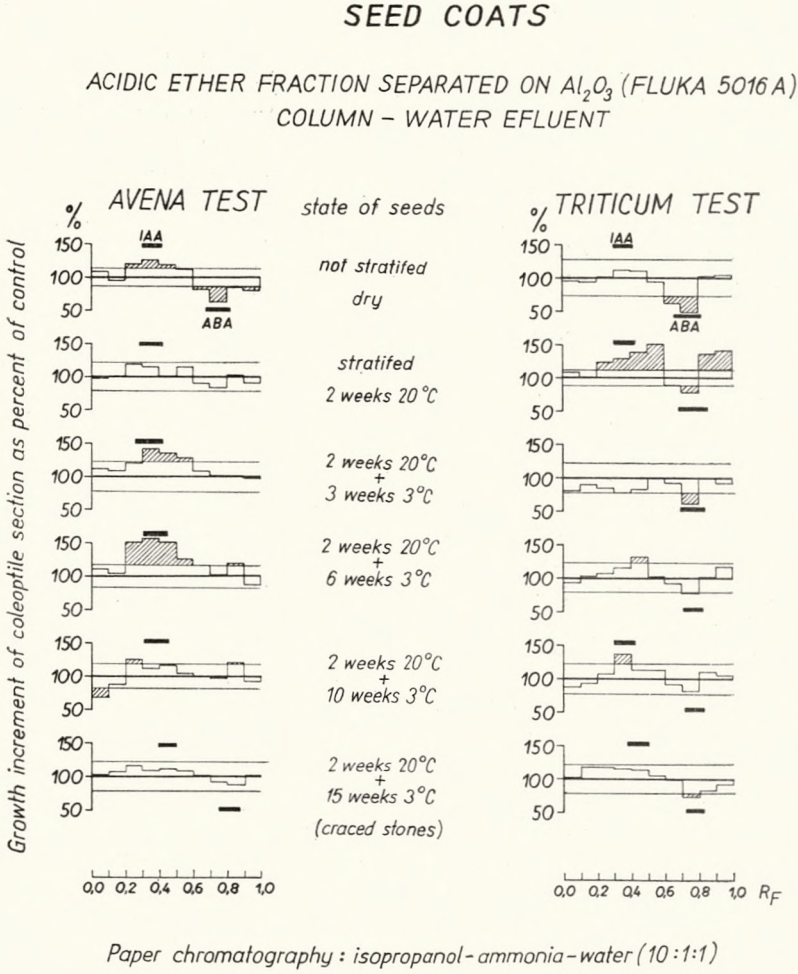

Fig. 8. Histograms of the bioactivity of the acidic ether fraction after separation on Al_2O_3 column (water effluent) from mazzard (*Prunus avium* L.) seed coats

The extracts have been chromatographed on Whatmann No. 3 filter paper, by isopropanol-ammonia -water (10 : 1 : 1) and segments of the chromatogram were bioassyed by the wheat coleoptile elongation tests (growth inhibitors) and by the oat coleoptile elongation test (growth promoters). Each histogram represent the activity of extracts from 60 seed coats. Horizontal lines mark off the least significant difference from controls (100%) at a confidence level of $p=0.95$

c) Auxin activity in ethanol eluate from Al_2O_3 (Fluka 5016 A) column

The neutral fraction after an appropriate chromatographic separation has been tested by the oat coleoptile segments bioassay. Results of these bioassays are presented in Fig. 9. For comparison the acid fraction is presented besides, in which the dominant auxin is probably IAA.

In the solvent studied by us the biological activity was found in three

ACIDIC ETHER FRACTION SEPARATED ON Al_2O_3 (FLUKA 5016 A) COLUMN

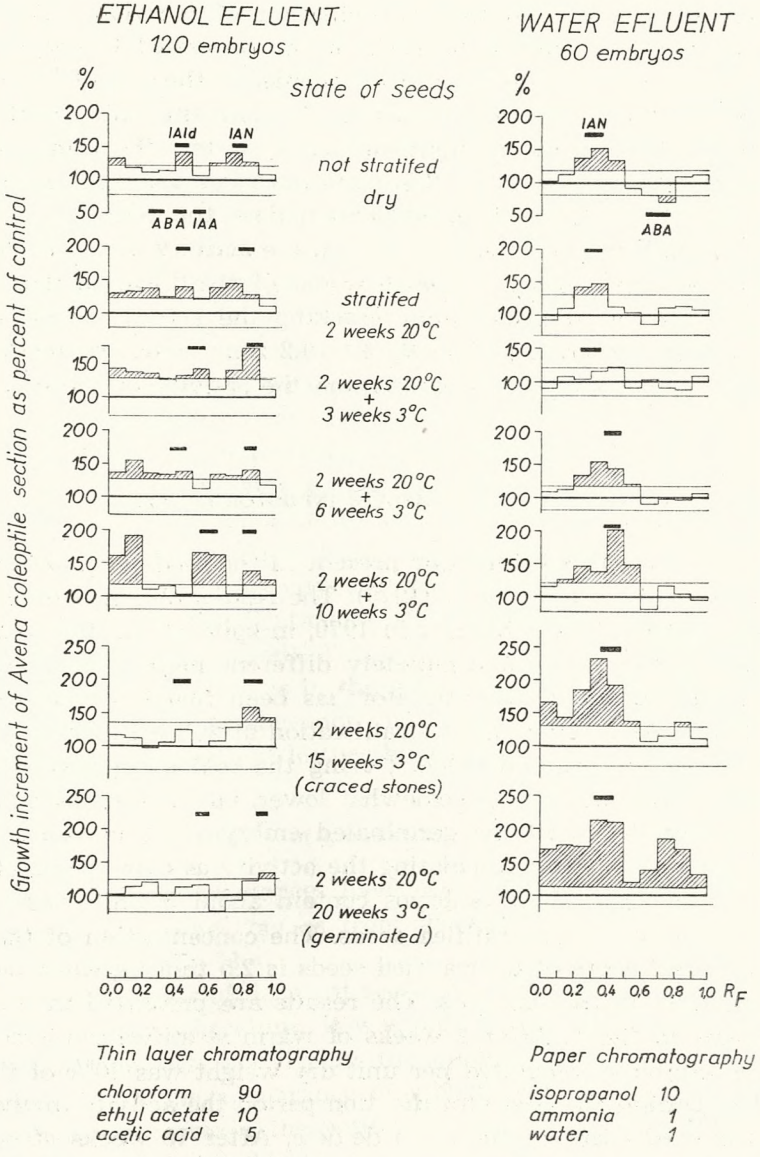

Fig. 9. Histograms of the bioactivity of the acidic ether fraction from mazzard embryos after separation an Al_2O_3 column (ethanol and water effluents)

The extracts (ethanol effluent) from 120 embryos has been chromatographed on TLC, by chloroform-ethyl acetate-acetic acid (90 : 10 : 5) and segments of the chromatogram after elution were bioassayed by the oat coleoptile elongation test. The water effluent from 60 embryos has been chromatographed on Whatmann No. 3 filter paper, by isopropanol-ammonia-water (10 : 1 : 1) and bioassayed by the oat coleoptile elongation test. Horizontal lines mark off the least significant difference from control (100%) at a confidence level of $p=0.95$

zones at: I R_F 0.1 - 0.2, II R_F 0.5 - 0.7 and III R_F 0.9 - 1.0. From the observations of Linser (1954) and on the basis of our own studies it appears that these substances are probably indole derivatives, indole-3-acetonitryle at R_F 0.9 - 1.0 and indole-3-acetaldehyde at R_F 0.5.

As regards the quantitative participation of individual substances during stratification, and on the basis of the presented histograms it is possible to observe certain regularities thanks to the use of always the same number of embryos for extraction. In dormant embryos the activity in these three zones is slight and more or less the same. After 2 weeks of stratification at 20°C this state does not undergo any significant change. Only after cold stratification does the activity in the R_F 0.9 - 1.0 zone increase and then decrease while activity in the R_F 0.1 - 0.2 zone increases continuously. After 10 weeks of stratification that is just before the stage of intensive stone cracking the activity of substances in this fraction was greatest, the R_F 0.1 - 0.2 zone being the most active one. In the further stages of stratification the activity of these substances declined.

3. ACTIVITY OF THE GROWTH INHIBITOR IN SEEDS

Studies on the growth inhibitor present in the seeds of mazzard have been conducted by K r a w i a r z (1970). The results obtained in the present study confirm those obtained in 1970, in spite of the fact that previously use was made of a completely different method of extraction. The maximal content of the inhibitor has been found in the dry dormant embryos. After 2 weeks of stratification at 20°C the activity drops to about 2/3 of the original value. During the cold stratification period the activity of the inhibitor is somewhat lower, but it remains at a more or less common level. In the germinated embryos the presence of the inhibitor was noted. After calculating the activity as equivalents of ABA per unit dry weight, these embryos contain about 5 times less of the inhibitor compared to unstratified seeds. The concentration of the inhibitor in the seed coats of unstratified seeds is 2.5 times greater per unit dry weight than in the embryos. The results are presented in the form of histograms in fig. 8. After 2 weeks of warm stratification concentration of the inhibitor calculated per unit dry weight was 20% of the original value. During the cold stratification period the activity of the inhibitor in the seed coats continues to decline. After 10 weeks of stratification at 3°C the inhibitor was no longer detectable.

In the stones (endocarps) the presence of the inhibitor was also found, which was similar in properties to the one found in the embryo and in the seed coats. However its activity in the stones was very small.

In the eluates from the Al_2O_3 column made with ethanol no inhibitor was found.

4. CONTENT OF GIBBERELLINS IN THE SEEDS OF MAZZARD

a) Activity of gibberellins in the embryos

In dry unstratified embryos substances are present which are active in the bioassay for gibberellins, the lettuce hypocotyl growth test. Results of biotests on extracts from embryos (acid and neutral fraction of ethyl acetate) are presented in Fig. 10. In the embryos there dominates a substance that takes up the position in the R_F zone of 0.1 - 0.2 following development in the solvent used. Activity in this zone increases during stratification. In germinated embryos it is 5 times as active as in the dormant embryos. After the warm stratification period activity has appeared in the R_F zone 0.6, where there was nothing in the dormant seeds, and again all activity disappears on transfer of the seed to cold stratification at 3°C. In embryos from cracked stones activity appears in the R_F zone 0.9. In germinated embryos the activity of this zone increases and a further active zone appears at R_F 0.5. In the extract from embryos as well as from the seed coats the presence of a substance was established which is toxic to lettuce seedlings and in the solvent used this substance remains at the starting point of the chromatograms.

In the neutral ethyl acetate fraction from unstratified embryos it was established by the biotest that the extract contains activity in the R_F zones 0.5 and 0.9. Activity of these substances disappears and only after 10 weeks of stratification at 3°C it reappears again at R_F 0.1 and R_F 0.4 (in the solvent used). This activity increases considerably in the next analysed stage, that is in the germinated embryos. Into the neutral fraction the toxic substance does not enter, and there are no zones of inhibition to the growth of lettuce hypocotyls.

b) Activity of gibberellins in the seed coats

Gibberellin like substances have been extracted from seed coats and analysed in the same way as from the embryos. Results are presented as histograms in Fig. 11.

In the seed coats of dormant seeds the acid ethyl acetate fraction did not contain any gibberellins. It was only in the seed coats from cracked stones that the first activity in the gibberellin biotest was noticed. It is probable that the appearance of gibberellins in that stage is associated with an increase in the activity of the seed coats, associated with the process of stone cracking.

In the neutral ethyl acetate fraction from the seed coats from seeds stratified for 2 weeks at 20°C activity was observed at the R_F zones 0.0 - 0.1 and 0.4. In the further stages no activity was found. In the extracts from seed coats from cracked stones the active zones are at R_F 0.1 and 0.6.

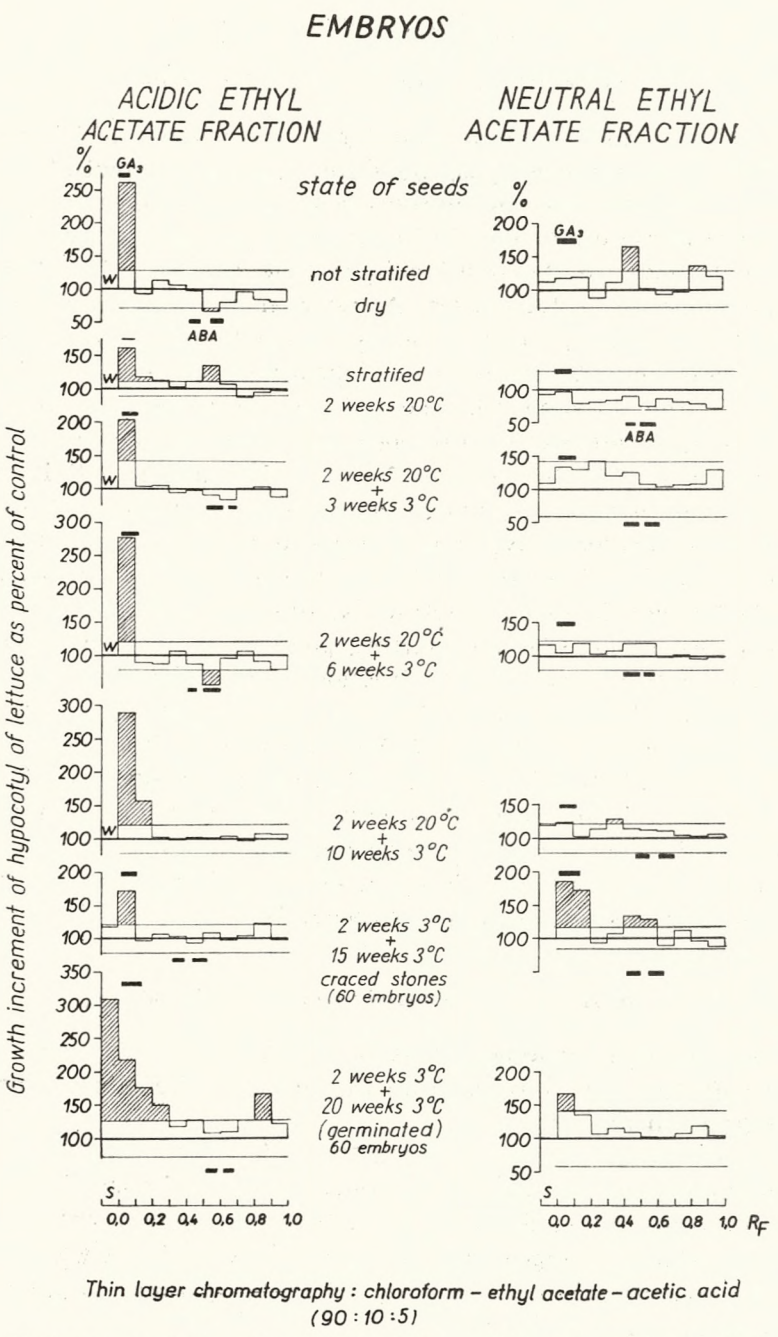

Fig. 10. Histograms of the bioactivity of the acidic and neutral ethyl acetate extracts from mazzard (*Prunus avium* L.) embryos

The extracts have been chromatographed on TLC by chloroform-ethyl acetate -acetic acid and bioassayed by the growth of hypocotyl of lettuce tests. Each histogram represents the activity of extracts from 120 embryos (60 embryos from cracked stones and germinated seeds). Horizontal lines mark off the least significant difference from controls (100%) at a confidence level $p=0.95$

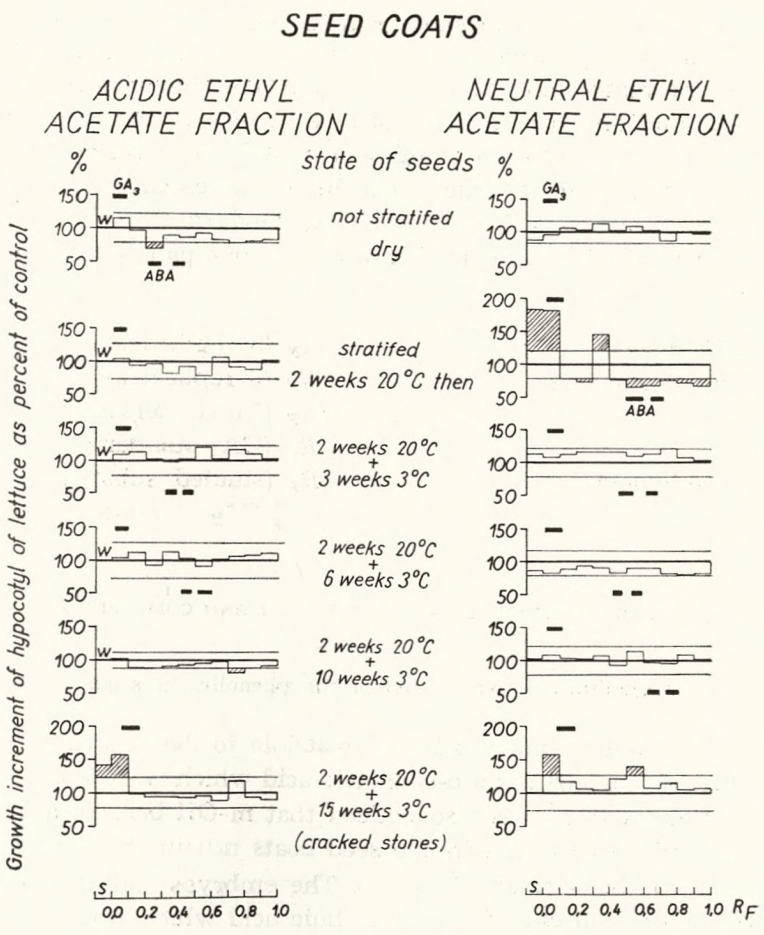

Fig. 11. Histograms of the bioactivity of acidic and neutral ethyl acetate extracts from mazzard (*Prunus avium* L.) seed coats

The extracts have been chromatographed on TLC by chloroform-ethyl acetate -acetic acid and bioassayed by the hypocotyl of lettuce tests. Each chromatogram represents the activity of extracts from 120 seed coats. Horizontal lines mark off the least significant difference from controls (100%) at a confidence level of $p=0.95$

c) Identification of gibberellin from the most active zone

In order to identify gibberellin from the most active zone for the extractions separate use was made of the unstratified seeds and germinated embryos. The acid ethyl acetate fraction was developed on thin layer plates in chloroform-ethyl acetate- acetic acid (60:40:5), after which the most active zone in stimulating lettuce hypocotyl growth was separated out. After elution of the substance contained there, the extract

was spotted onto a Wh 3 chromatographic paper and developed two directionally in 1) isopropanol-ammonia-water (10:1:1) and 2) 3% acetic acid. The chromatograms were developed colorimetrically with 5% H_2SO_4 in ethanol and heated in a hot stream of air they were observed under a quartz lamp with an emmission at 365 nm wavelength. On the chromatograms a characteristic green-blue fluorescence was observed similar to that which was given by the GA_3 standard.

The values of R_F were as follows: in isopropanol-ammonia-water (10 : 1 : 1)

unstratified seeds	R_F (3 replicates)	0.49
germinated embryos	R_F (3 replicates)	0.50
	R_F (from literature)	0.52
	R_F (GA_3 standard)	0.50
in 3% acetic acid	R_F (studied substance)	1.0
	R_F (GA_3 standard)	1.0

5. PHENOLIC COMPOUNDS — DISTRIBUTION AND COMPOSITION

a) Qualitative composition of the phenolics in seeds

In the germinated embryos it was possible to demonstrate (Table 4) the presence of coumarin and o-coumaric acid which were not detectable in dormant embryos. It was also noticed that m-OH benzoic acid is present in the embryos but not in the seed coats nor in the stones. It was present only in the dormant embryos. The embryos and the seed coats differ also in the content of protocatechuic acid which was absent from the embryos.

In the stone shells of mazzard seeds aldehyde derivatives from benzoic and p-coumaric acids and free phenolic acids have been found. The composition of the phenolics in the stones has not changes much during stratification.

b) Content of chlorogenic acid

Quantitative changes in the content of chlorogenic acid in embryos and seed coats are presented in Fig. 12. The course of the chlorogenic acid content indicates the direction in which the phenolic compounds change in embryos during stratification. It was possible to confirm this also chromatographically. In the early stratification period the level of phenols in the embryos does not undergo major changes. A basic acceleration in the turnover of phenolic compounds takes place during stone cracking and in the germinated embryos.

Table 4

Phenolic substances contained in the extracts from embryos, seed coats and stone shells of mazzard (*Prunus avium* L.) seeds

\+ present — absent

Phenolic substances	Embryos		Seed coats		Stone shells	
	unstratified	germinated	unstratified	germinated	unstratified	germinated
1. *p*-coumarylquinic acid	+	+	+	+	–	–
2. chlorogenic acid	+	+	+	+	–	–
3. naringenin	+	+	+	+	–	–
4. quercetin	+	+	+	+	–	–
5. *p*-coumaric acid	+	+	+	+	+	+
6. *o*-coumaric acid	–	+	–	–	–	–
7. coumarin	–	+	–	–	–	–
8. caffeic acid	+	+	+	+	+	+
9. ferulic acid	+	+	+	+	+	+
10. synapic acid	–	+	–	–	–	–
11. *p*-OH benzoic acid	+	+	+	+	+	+
12. *m*-OH benzoic acid	+	–	–	–	–	–
13. salicylic acid	+	+	+	+	–	–
14. vanillic acid	+	+	+	+	+	+
15. syringic acid	+	+	+	+	+	+
16. protocatechuic acid	–	–	+	+	+	+
17. gentisic acid	+	+	+	+	+	+
18. *p*-OH benzoic aldehyde	–	–	–	–	+	+
19. vanilin	–	–	–	–	+	+
20. syringic aldehyde	–	–	–	–	+	+
21. coniferylic aldehyde	–	–	–	–	+	+
22. quercetin flavonoids	–	+	–	–	–	–

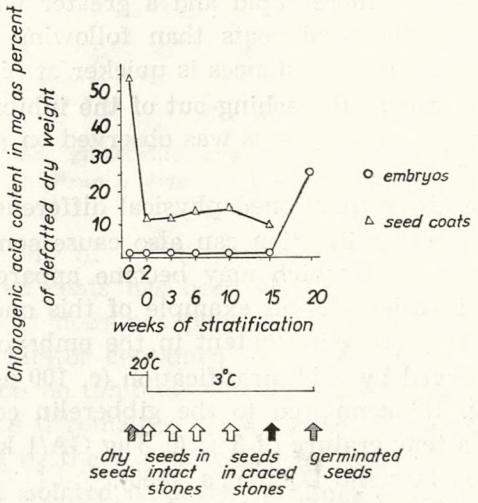

Fig. 12. Changes in chlorogenic acid content in mazzard seeds (*Prunus avium* L.) in mg as percent of defatted dry weight

In the seed coats of unstratified seeds there is much more chlorogenic acid. After two weeks of stratification at 20°C the level drops to become 5 times lower, and then remains more or less constant until the end of stratification.

IV. DISCUSSION

During a warm-followed-by-cold stratification of mazzard seeds, the pits crack between the 10th and 15th week and germination starts between 15th and 20th week. During a cold stratification the dates of onset of stone cracking and germination are similar, however the number of cracked and germinated seeds after 20 weeks was 4 time smaller than in the case of the warm-followed-by-cold stratification (K r a w i a r z, 1970). From the germinated seeds normal seedlings develop regardless of the stratification method.

The problem of dormancy and germination in the seeds of mazzard is associated with the following questions:

1. What is the effect of the warm stratification period on dormancy breaking in the seeds of mazzard?
2. What is the role of an inhibitor in seed dormancy?
3. What is the role of the seed coats?
4. Are the relative concentrations of various growth substances in a causative relation with seed dormancy?

In order to be able to reply to the first question, one has to analyse the differences which occur between seeds stratified by the warm-followed-by-cold and by the cold method. In the conditions of the warm-followed-by-cold stratification it was observed that during the first two weeks there was both a more rapid and a greater water imbibition by the embryos and by the seed coats than following cold stratification only. Since the diffusion of substances is quicker at higher temperatures one can expect a more rapid washing-out of the inhibitor both from the seed coats and from the embryos as was observed on peaches by W o n g (1971).

Apart from the above mentioned physical differences concerning the diffusion of heat, the stratification can also cause some metabolic reactions, the consequences of which may become apparent only later during the cold stratification. As an example of this one can mention the several times higher gibberelin content in the embryos of seeds subjected to a warm-followed-by-cold stratification (c. 100 μg GA_3/1 kg of dry weight — see Fig. 13) compared to the gibberelin content in the embryos statified at a temperature of 3°C (c. 8 μg GA/1 kg of dry weight — compare with P r o c t o r and D e n n i s, 1968).

The second question concerns the role of the inhibitor in the dormancy of mazzard seeds.

Seed coats and embryos of non-stratified seeds contain considerable quantities of the inhibitor, abscisic acid. In the seed coats on a per dry weight basis there is 2.5 times more of the inhibitor than in the embryos (see Fig. 14). It occurs primarily in a soluble form and is readily washed out with water, both in the moist stratification medium and in distilled

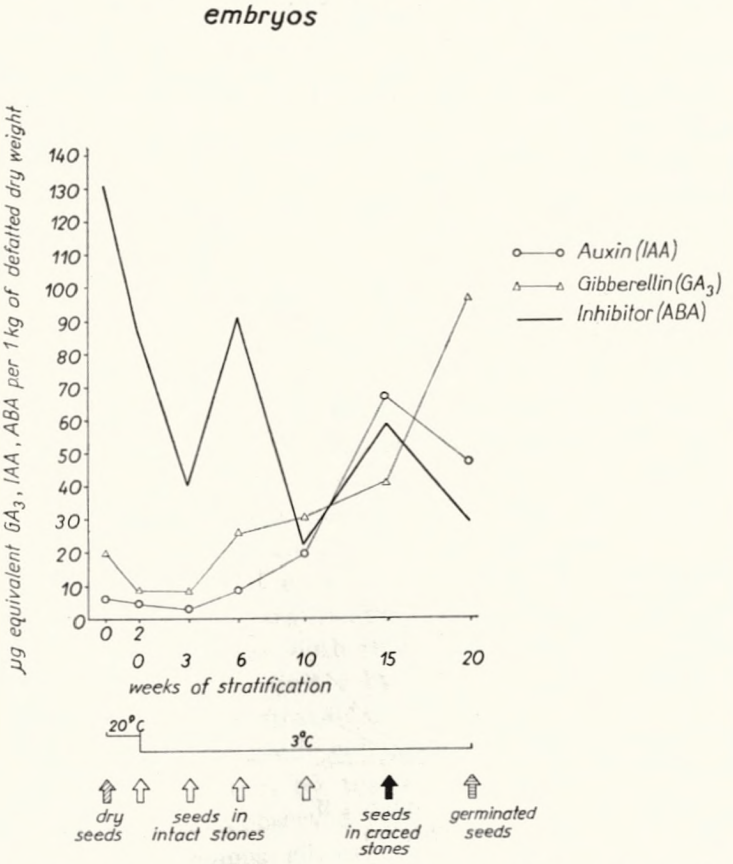

Fig. 13. Change of auxin, gibberellin and growth inhibitor content in mazzard embryos (*Prunus avium* L.) during stratification

water at a temperature of +3°C. Since the majority of the inhibitor contained in the seed coats has been washed out already in the early stratification period it does not play such a role in the regulation of dormancy as the inhibitor contained in the embryos. After two weeks of warm stratification no inhibitor was detected in the living seed coats, while in the embryos it remained for a further 12 weeks (K r a w i a r z, 1970) and this could be the reason why seed in this stage do not germinate and why from isolated embryos seedlings develop with annomalities. Some fluctuations in the level of the inhibitor in the embryos (Fig. 13) tend to suggest that the inhibitor occurs in a bound form, biologi-

cally inactive, and that it is being gradually released as dormancy is being broken.

The presence of the inhibitor in the embryos could be the cause of the inhibition of enzyme activity. It is known that abscisic acid inhibits the biosynthesis of nucleic acid (Overbeek, 1966; Khan and Heit, 1969), and of enzymes, amylases (Paleg, 1960) and proteases Varner and Johri, 1968).

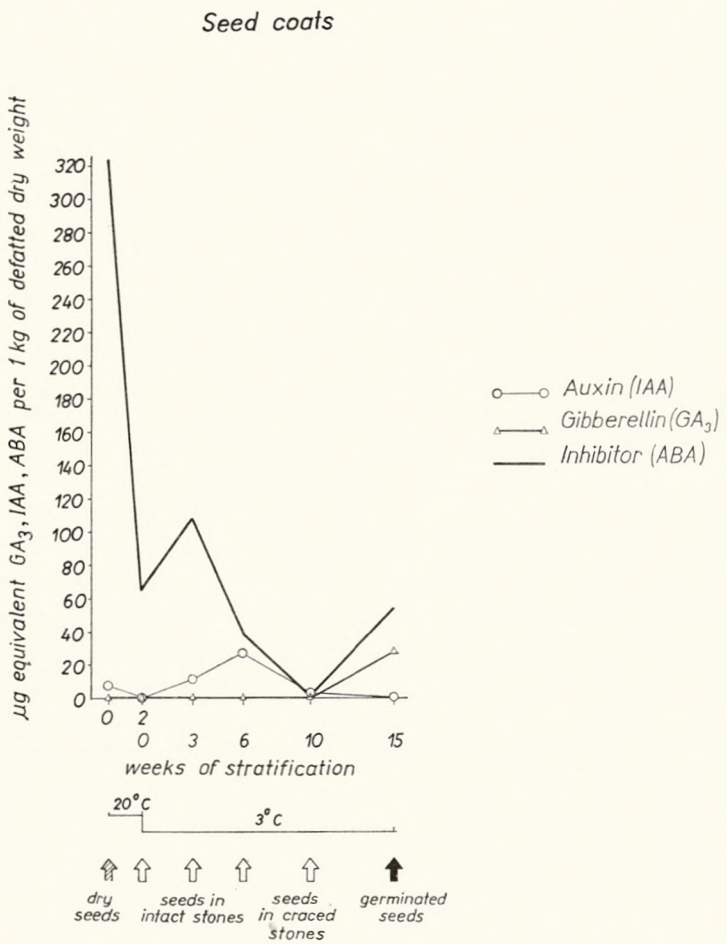

Fig. 14. Changes of auxin, gibberellin-like substances and growth inhibitor in seed coats of mazzard (*Prunus evium* L.) seeds during stratification

The next problem is the role of seed coats, which is associated with their function in controlling oxygen penetration to the embroys and inhibitor diffusion from the embryos to the stratification medium.

The seed coats can by their very presence inhibit diffusion of the inhibitor from the embryo to the stratification medium and conversly the access of water and oxygen to the embryo. The question of water

uptake and inhibitor diffusion have already been discussed, while the question of oxygen uptake by the embryos and the role played here by the seed coats is a separate problem.

Isolated embryos absorb more oxygen than embryos within the seed coats (Fig. 5). Côme (1967) who has observed a similar phenomenon believes that the phenolic substances present in the seed coats, primarily chlorogenic acid and *p*-coumarylquinic acids oxidize and thus limit the movement of oxygen to the embryos. In the seed coats and in the stone shells of mazzard considerable quantities of free phenolic acids and aldehyde derivatives of benzoic and cynammic acids have been found (Table 4). Judging by the intensity of fluorescence of the spots of phenolic compounds at the beginning and end of the stratification the content of phenolic substances has not changed. A constant level of chlorogenic acid in the seeds coats (Fig. 12) also does not suggest that it underwent oxidation during stratification. Only during the period of warm stratification it is somewhat washed out similarily as the inhibitor, but later its level is maintained constant to the end of stratification time.

Pollock and Olney (1959), Ollney and Pollock (1960) who have also studied the seeds of mazzard have tried to explain the cause of dormancy of these seeds through an investigation of the energy effectiveness of the respiration process. They have stratified the seeds at 5°C and at 25°C and studied the effect of dinitrophenol a substance that uncouples the oxidative process from phosphorylation on respiration. Dinitrophenol has caused a considerable increase in the respiration rate at the onset of the cold stratification. This effect declines as the stratification progresses. These authors believe that in dormant seeds the intensity of respiration is limited by a lack of ADP, which is gradually being accumulated during stratification.

The main role of the seed coats is played during stone cracking. An increased water imbibition by the seed coats treated with gibberellin (GA_3) has been already observed after 24 hours. This suggests that the appearance of endogenous gibberellins in the seed coats during stone cracking is in a causal relationship with the increased water-logging of the tissues. Stone cracking, the growth of the radicle and the increase in gibberellin and water content of the embryos all come simultaneously.

The next question concerns the role of growth regulators. Do the mutual relations of the inhibitor, auxin and gibberellins have a causal relation the dormancy and germination of seeds?

It appears that, such a relation can be found. Seed dormancy is associated with a high level of the inhibitor (abscisic acid). As was mentioned above abscisic acid inhibits the biosynthesis of nucleic acids and enzymes. As the dormancy is being broken the proportion of the inhibitor (ABA) to the auxin (IAA) and gibberellin (GA_3) declines and

this is paralleled by an increase in the biosynthesis of nucleic acids and enzymes.

Pillay and Edgerton (1965) treating seeds of mazzard with a solution of GA_3 have established an increase in the content of auxin in the embryos and a hestening of germination. In the optimal conditions of stratification an increase in the level of endogenous gibberellin has been accompanied by an increase in the level of auxin (Fig. 13). The highest level of auxin was found at the time of stone cracking. Germinating embryos have less auxin (Fig. 13). It is probably being utilized by the beginning process of growth.

Proctor and Dennis (1968) have studied gibberellin in the seeds of mazzard stratified at 3°C. They have established that the gibberellin contained in dormant seeds does not undergo any changes during stratification. In the conditions of warm, followed-by-cold stratification the final level of gibberellin was almost 20 times higher than these authors reported. In germinated embryos there is 5 times more gibberellin than in dry unstratified embryos (Fig. 13). Gibberellins induce a biosynthesis of amylases and proteases (Varner and Johri, 1968) and this induction is being antagonized by abscisic acid. During the stage of stone cracking and germination of mazzard seeds a considerable decline in the level of proteins and fats was observed (Fig. 4, Table 1), while the content of micromolecular substances increases. This must result in an increase of the osmotic pressure and water absorption by the seed coats and embryos.

Thus one can imagine the following stages of dormancy breaking:

1. warm stratification reduces the inhibitor content, which facilitates;
2. synthesis of gibberellins during cold stratification; these are needed to;
3. induce synthesis of catabolic enzymes which;
4. mobilize the reserve substances into a soluble form, thereby;
5. raising the osmotic pressure and leading to;
6. water absorption by the seed coats and embryo, with the result that;
7. stones crack and;
8. oxygen access to embryos is facilitated;
9. permitting the growth of radicle and plumule.

V. CONCLUSIONS

1. In dry unstratified embryos about 35% of fats and 5,5% of protein nitrogen was found together with a small quantity of chlorogenic acid. They contain free phenolics: *p*-coumarylquinic, *p*-coumaric, caffeic, ferulic, *p*-OH benzoic, *m*-OH benzoic, salicylic, vanillic, gentisic and syringic acids and the flavonoids quercetin and naringenin. In dry unstra-

tified embryos the presence of auxins, gibberellins and an inhibitor have been demonstrated. In the seed coats from unstratified seeds there are present besides the m-OH benzoic acid the same phenolics as in the embryos. Besides there is free protocatechuic acid in the seed coats. On a per dry weight basis the content of chlorogenic acid is 50 times greater in the seed coats than in the embryos. Similarily there is also 2.5 times more of the inhibitor. Small quantities of auxins were found there also but there were no gibberellins in the seed coats.

In the stone shells of unstratified seeds considerable quantities of phenolic acids have been identified, namely *p*-coumaric, caffeic, ferulic, *p*-OH benzoic, gentisic, vanillic, syringic, protocatechuic and the aldehydes, *p*-OH benzoic, vanillin, syringic and coniferylic. In the stone shells an active inhibitor was found, identical with the one which was present in the embryos and in the seed coats, though in minimal quantities.

2. During stratification for 2 weeks at 20°C the embryos absorbed considerable quantities of water. The embryos in terms of water content and oxygen consumption have attained a level comparable to that in embryos from seeds freshly collected from trees. In that period no changes in the content of fats, proteins or chlorogenic acid were observed. Also the composition of the phenolics has not changed. After 2 weeks of warm stratification a considerable drop was observed in the content of the inhibitor (by about 1/3 of the initial value). No changes were observed in the content of the auxins and in the gibberellins.

In the seed coats from seeds stratified for 2 weeks at 20°C a much greater drop in the level of the inhibitor was observed (by about 80%). Also the level of chlorogenic acid drops substancially. The amount of fats does not undergo any major changes. In seed coats during that time neither auxins nor gibberellins were found. In the neutral ethyl acetate fraction substances were found that were active in the gibberellin bioassay.

3. During the cold stratification period no changes were observed (from the moment of transfering the seeds from 20°C to 3°C up to 10 weeks of stratification) as regards water content, oxygen absorption, content of storage substances (fats, proteins) and content of chlorogenic acid. The changes were greatest in the content of growth regulating substances. The amount of inhibitors in the embryos is subjected to considerable fluctuations but to a much lower degree than in the unstratified embryos. The content of gibberellins in that period increases most. Also the quantity of auxins increases. After 10 weeks of the cold stratification the greatest activity was observed in the tests for auxins from the neutral fraction that presumably contains auxin precursors.

In the seed coats a gradual increase in the water content was observable. There were no changes in the content of fats and of chlorogenic

acid. A further decline in the level of the inhibitor was noted. The seed coats do not contain any gibberellins, while the level of the auxins increases and reaches a maximum after 10 weeks of cold stratification.

4. After 15 weeks of stratification in the cold conditions seeds from cracked stones have been analysed. In the embryos from these seeds a slight increase in the water content was observed. The absorption of oxygen by the embryos increases 3 times. Embryos from uncracked stones absorb oxygen at an unchanged level. There is a slight increase of the fraction soluble in petroleum ether. In embryos from this stage the highest content of auxin was noted. The content of gibberellin in the embryos from cracked stones declines. In these embryos the lowest levels of the inhibitor were noted. In the stone bursting process a considerable role is probably played by the seed coats. In that stage the water content of the seed coats increases substancially, by about 15%. In seed coats from cracked stones there appears in the acid ethyl acetate fraction a gibberellin. In the neutral ethyl acetate fraction also substances were found that were active in the bioassay using growth of lettuce hypocotyls. In the seed coats from that stage the presence of auxins was not noted.

5. In the germinated embryos very substancial changes were observed in all the processes studied. The water content increases by about 15%. A rapid dissimilation of reserve substances results (fats, proteins), while at the same time the fraction soluble in 80% methanol and 70% acetone increases substancially (micromolecular substances). Probably associated with these processes is the 5-fold increase in the uptake of oxygen by the embryo. Embryos from uncracked stones absorb oxygen at an unchanged level.

In the germinated embryos the content of chlorogenic acid increases and new phenolics appear: o-coumaric acid, coumarin, synapic acid and numerous glucosides of quercetin. In the germinated embryos the highest content of gibberellins was observed. In the acid ethyl acetate fraction there is an increase in the content of gibberellin that has first appeared at the R_F zone 0.8 - 0.9 during the previous test period. In the neutral ethyl acetate fraction activity declines somewhat compared to the state during the previous test period.

In the germinated embryos there is an inhibitor present with the same properties as the beginning of stratification but in considerably lower amounts. Also compared to the previous test period there is a decline in the level of the auxin.

6. On the basis of the behaviour in the biotest, the chromatographic studies and the chemical tests the main active substance in the lettuce hypocotyl biotest is probably the gibberellic acid GA_3.

7. During the breaking of dormancy of mazzard seeds there is an increase in the level of gibberellins in the embryos.

8. The chromatographic observations and the behaviour in the biotests indicate that the main auxin present in the mazzard seeds is the indole-3-acetic acid. The content of this auxin in the stratified embryos increased and the highest levels were found in embryos from cracked stones.

9. The results obtained in the present study on the presence of an inhibitor confirm the results reported in 1970 (K r a w i a r z, 1970). The better method of extracting this inhibitor from the seeds has permitted a demonstration of its presence even in germinated embryos. Also it was confirmed that the level of this inhibitor declines as the dormancy is being broken.

10. The substances present in the ethanol eluate from an Al_2O_3 Fluka 5016 A column and active in the oat coleoptile extension bioassay are presumably natural precursors of IAA. On the basis of chromatographic observations it is suspected that presumably these substances are an aldehyde of indole-3-acetic acid and indole-3-acetonitrile.

11. The process of dormancy breaking in embryos is characterized by dynamic changes in the content of growth substances. In cracked stones, in which the dormancy has already been broken, active physiological processes begin: an increase in water content, dissimilation of reserve substances, increase in oxygen consumption, increase in the content of phenolics, while at the same time there is an increase in gibberellins and a high level of auxins. The level of the inhibitor is low at that stage.

12. The role of seed coats in the dormancy of seeds is twofold. They constitute a mechanical and a physiological barrier. In the process of stone cracking they participate by mechanically bursting the stone, and through a physiological stimulus they appear to initiate the activity of the embryo.

13 .The stone shell constitutes a mechanical barrier and a biological one separating the embryo from the efects of the environment. It makes it possible for the embryo to undergo the endogennous processes of dormancy breaking. The shells constitute also a physiological barrier (they reduce the absorption of oxygen). Probably they also represent a fungistatic barrier in view of the fact that they contain free phenolic acids and aldehydes of phenolic acids.

SUMMARY

In various stages of the warm-followed-by-cold stratification (2 weeks at 20°C and then at 3°C — stone cracking after 15 weeks, germination after 20 weeks) of mazzard seeds, the content of endogenous growth substances was investigated (auxins, gibberellins, inhibitors) as well as the uptake of water and oxygen and the content of fats, proteins and phenolic substances.

Dormancy of mazzard seeds is associated with the presence of the inhibitor,

abscisic acid. After 2 weeks of the warm stratification the content of abscisic acid in the embryos drops to 70% and in the seed coats to 20% of the initial value, while after germination the values are respectively 22% and 17%.

During the cold stratification period an increase in the content of auxins (IAA) and gibberellins (GA_3) was observed reaching respectively a 3 and a 5 times greater level on germination than was in the dry seeds. As the after ripening process progresses during stratification the ratio of the inhibitor to auxins or gibberellins declines in the embryos. In the seed coats of dormant seeds, gibberellin is absent and appears only in the waterlogged seed coats during stone cracking, when auxins are completely absent.

During warm and cold stratification the level of water content, fats, proteins and oxygen absorption, do not undergo major changes. In the embryos of germinating seeds there is a rapid increase in water content, the levels of fats and proteins decline and the oxygen absorption is 5 times the value for dormant seeds.

The embryos contain the following phenolic acids: *p*-coumaric, *p*-coumarylquinic, chlorogenic, ferulic, *p*-OH benzoic, m-OH benzoic, salicylic, vanillic, gentisic, syringic as well as naringenin and quercetin. In the seed coats the same phenolics were present except *m*-OH benzoic acid, but with protocatechuic acid. Seed coats contain a 50 times greater concentration of chlorogenic acid than the embryos. Already after the warm stratification period, it dropped to 20% of the initial value and is later maintained at this level. In the embryos an increase in the level of chlorogenic acid was observed only during germination. In that stage new phenolics appear in the embryos: *o*-coumaric and synapic acids, coumarin and numerous glucosides of quercetin.

Embryos in the stones absorb less oxygen than when isolated. In the stone shells the presence of the following phenolic acids was observed: *p*-coumaric, ferulic, protocatechuic, *p*-OH benzoic, vanillic, syringic and their respective aldehydes, however it does not appear that their presence is related to the reduced oxygen absorption by intact seeds.

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KAZIMIERZ KRAWIARZ

Dynamika endogennych substancji wzrostowych i regulacja spoczynku nasion dzikiej czereśni (Prunus avium L.)

Streszczenie

Spoczynek nasion dzikiej czereśni ustępuje podczas stratyfikacji przez 2 tygodnie w temperaturze 20°C, następnie 3°C, która jest dla nich optymalna.

W różnych stadiach stratyfikacji badano zawartość endogennych substancji wzrostowych (auksyn, giberelin i inhibitora) oraz inne wskaźniki stanu fizjologicznego, jak pobieranie wody, pobieranie tlenu, zmiany zawartości tłuszczów, białek i fenoli.

Suche, spoczynkowe zarodki dzikiej czereśni zawierają najwyższy poziom inhibitora, prawdopodobnie kwasu abscysynowego. Po 2 tygodniach stratyfikacji w 20°C zawartość tego inhibitora spada o 1/3 początkowej zawartości. Suche, nie stratyfikowane zarodki (około 6,5% wody) zawierają 35% tłuszczów, 5,5% azotu białkowego, niewielką ilość kwasu chlorogenowego. W tych zarodkach obecnie są auksyny i gibereliny. Zarodki te pobierają minimalne ilości tlenu. Zawierają one wolne fenole — kwasy: *p*-kumarowy, *p*-kumaroilochinowy, kawowy, ferulowy, *p*-OH benzoesowy, *m*-OH benzoesowy, salicylowy, waniliowy, gentyzynowy, syryngowy, naringeninę i kwercetynę.

Okrywy nasienne z suchych, nie stratyfikowanych nasion (6,5% wody) zawierają 2,5 razy więcej inhibitora (w przeliczeniu na suchą masę) aniżeli zarodki. Wykryto w nich niewielką ilość auksyn, nie ma w nich giberelin. Spadek zawartości inhibitora w okrywach nasiennych jest gwałtowniejszy niż w zarodkach. Po 2 tygodniach ciepłej stratyfikacji stężenie inhibitora wynosi 20% stężenia wyjściowego. W suchych nie stratyfikowanych okrywach nasiennych jest około 13% tłuszczów i prawie 50 razy więcej kwasu chlorogenowego niż w zarodkach (w jednostce suchej masy). Pod względem składu jakościowego fenoli większych różnic nie wykryto. W okrywach nasiennych nie wykryto kwasu *m*-OH benzoesowego, zawierają one natomiast kwas protokatechusowy.

Zarodki bez okryw nasiennych pobierają znacznie więcej tlenu aniżeli całe pestki. Skorupki nasion w dużym stopniu zmniejszają pobieranie tlenu. W skorupkach nie stratyfikowanych nasion zidentyfikowano znaczne ilości fenolokwasów: *p*-kumarowy, kawowy, ferulowy, *p*-OH benzoesowy, waniliowy, syryngowy, protokatechusowy oraz aldehydy: *p*-OH benzoesowy, waniliowy, syryngowy i koniferylowy. Ze skorupek wyekstrahowano aktywny inhibitor o właściwościach podobnych do tego, który jest obecny w zarodkach i okrywach nasiennych; występuje on jednak w minimalnych ilościach.

Po 2 tygodniach stratyfikacji w 20°C zarodki pod względem uwodnienia i pobierania tlenu osiągnęły stan, jaki występuje w zarodkach nasion świeżo zebranych z drzewa. W okrywach nasiennych obserwowano 80% spadek zawartości inhibitora. Zmniejsza się również zawartość kwasu chlorogenowego.

W chłodnym okresie stratyfikacji nie obserwowano większych zmian pod względem uwodnienia, pobierania tlenu przez zarodek, zawartości tłuszczów, białek, a również fenoli. W tym okresie obserwowano znaczny wzrost zawartości stymulatorów wzrostu: auksyn i giberelin.

W zarodkach z pękniętych pestek obserwowano znaczny wzrost aktywności auksyn. W tym okresie obserwowano najniższy poziom zawartości inhibitora. Zarodki pobierają 3-krotnie więcej tlenu aniżeli zarodki z pestek nie pękniętych. Zmiany pod względem uwodnienia, zawartości tłuszczów i białek są jeszcze nie-

wielkie. W okrywach nasiennych nasion z pestek pękniętych pojawia się aktywna giberelina. Nie ma w nich auksyn ani inhibitora. Wydaje się, że istnieje związek pomiędzy pojawieniem się tej gibereliny i wzrostem uwodnienia.

Najgłębsze zmiany fizjologiczne występują w skiełkowanych zarodkach. Uwodnienie zarodków wzrasta o 15%. Następuje gwałtowna dysymilacja substancji zapasowych (spadek zawartości tłuszczów i białek). Równocześnie obserwowano wzrost frakcji rozpuszczalnych w 80% metanolu i 70% acetonie. Zarodki, w porównianiu z zarodkami z nieskiełkowanych nasion, pobierają w tych samych warunkach 5 razy więcej tlenu. Znacznie wzrasta w nich zawartość kwasu chlorogenowego. Pojawiają się nowe związki fenolowe: kwas *o*-kumarowy, kumaryna, kwas synapinowy, liczne glukozy kwercetyny. W skiełkowanych zarodkach obserwowano najwyższą zawartość gibereliny. Obecny jest także inhibitor. Skiełkowane zarodki w przeliczeniu na suchą masę zawierają 5 razy mniej tego inhibitora aniżeli zarodki z nasion nie stratyfikowanych. W tych zarodkach zmniejsza się ilość auksyn, wiąże się to z dużym zapotrzebowaniem tej substancji w procesie wzrostu.

Spoczynek nasion dzikiej czereśni wiąże się z obecnością inhibitora. Podczas stratyfikacji, w miarę ustępowania spoczynku, zmniejsza się stosunek zawartości inhibitora do zawartości stymulatorów. Ustępowanie spoczynku nasion wiąże się ze wzrostem zawartości endogennych stymulatorów wzrostu auksyn i giberelin.

Giberelina, która pojawia się w okrywach nasiennych w stadium pękających pestek, prawdopodobnie bierze udział w regulacji uwodnienia i aktywności tych okryw oraz bielma w procesie pękania pestek.

Na podstawie testów biologicznych oraz prób chromatograficznych wykazano, że dominującą auksyną jest prawdopodobnie IAA. Prócz tej auksyny są obecne w zarodkach jeszcze inne substancje aktywne w testach na auksyny, które na podstawie rozpuszczalności można by określić jako kwaśne i neutralne.

Dominującą gibereliną jest prawdopodobnie GA_3. Kwaśne i neutralne gibereliny nie były identyfikowane. W nasionach dzikiej czereśni występuje jeden inhibitor, który swym zachowaniem i właściwościami przypomina kwas abscysynowy.

КАЗИМЕЖ КРАВЯЖ

Динамика эндогенных ростовых веществ и регулирование покоя семян дикой черешни (Prunus avium L.)

Резюме

Покой семян дикой черешни прекращается во время стратификации в течение 2 недель при температуре 20°Ц, потом 3°Ц, которая для них является оптимальной.

В разные стадии стратификации исследовалось содержание эндогенных ростовых веществ (ауксин, гиббереллин и ингибитора) а также иных показателей физиологического состояния как: поглощение воды, усваивание кислорода, изменения содержания жиров, белка и фенолов.

Сухие, покоящиеся зародыши дикой черешни содержат самый высокий уровень ингибитора, вероятно, абсцисиновой кислоты. После 2 недель стратификации в 20°Ц содержание этого ингибитора уменьшается на 1/3 начального количества. Сухие не стратифицированные зародыши (около 6,5% воды) содержат 35% жиров, 5,5% белкового азота, небольшое количество хлорогеновой кислоты. В этих зародышах находились ауксины и гиббереллины. Зародыши эти поглощают в минимальных количествах кислород. Они содержат свободные фенолы — кислоты: *p*-кумаровую, *p*-кумарилохинную, кафейную, феруловую, *p*-OH бензойную, *m*-OH бензойную, салициловую, ванильную, гентизиновую, сиринговую, нарингенин и кверцетин.

Семенные оболочки из сухих, не стратифицированных семян (6,5% воды) содержат 2,5 раза больше ингибитора (в пересчёте на сухую массу), чем зародыши. Обнаружено в них небольшое количество ауксин, нет в них гиберелин. Уменьшение содержания ингибитора в семенных оболочках более резкое, чем в зародышах. После 2 недель тёплой стратификации концентрация ингибитора составляет 20% начальной концентрации. В сухих не стратифицированных семенных оболочках находится около 13% жиров и почти в 50 раз больше хлорогенной кислоты, чем в зародышах (в единице сухой массы). В отношении качественного состава фенолов не обнаружено большей разницы. В семенных оболочках не обнаружено *m*-OH бензойной кислоты, но они содержат прокатехусную кислоту. Зародыши без семенных оболочек поглощают значительно больше кислорода, чем целые косточки. Скорлупки семян в большой степени уменьшают поглощение кислорода. В скорлупках не стратифицированных семян идентифицированы значительные количества фенолокислот: *p*-кумаровая, кофейная, феруловая, *p*-OH бензойная, ванилиновая, сиреневая, протокатехусная и альдегиды: *p*-OH бензойный, ванильный, сиреневый и конифериловый. Из косточек был экстрагирован активный ингибитор с особенностями, похожими на те, которые находятся в зародышах и семенных оболочках; однако он выступают в минимальных количествах.

После двухнедельной стратификации в 20°Ц зародыши в отношении гидратации и поглощения кислорода достигали такого состояния, которые выступает в зародышах свежесобранных с дерева семян. В это время уменьшается содержание ингибитора. В семенных оболочках наблюдалось 80% уменьшение содержания ингибитора. Уменьшается также содержание хлорогеновой кислоты.

В холодный период стратификации не наблюдалось больших изменений в отношении гидратации, поглощения кислорода зародышем, содержания жиров, белков и фенолов. В этот период наблюдалось значительное увеличение содержания стимуляторов роста: ауксин и гиббереллин.

В зародышах из треснутых косточек наблюдался значительный рост активности ауксин. В этот период наблюдался самый низкий уровень содержания ингибитора. Зародыши употребляют в три раза больше кислорода, чем зародыши из косточек не треснувших. Изменения в отношении гидратации, содержания жиров и белков остаются ещё незначительными. В семенных оболочках из треснувших косточек появляется активный гиббереллин. Нет в них ауксин и ингибитора. Кажется, что существует связь появления этого гиббереллина с ростом гидратации.

Наибольшие физиологические изменения выступают в проросших зародышах. Гидратация зародышей увеличивается на 15%. Наступает резкая диссимиляция запасных веществ (уменьшение содержания жиров и белков). Одновременно наблюдался рост фракций, растворяемых в 80% метанола и 70% ацетона. Зародыши, в сравнении с зародышами из непроросших семян, поглощают в тех же условиях в 5 раз больше кислорода. Значительно увеличивается в них содержание хлорогеновой кислоты. Появляются новые соединения фенолов: *o*-кумаровая кислота, кумарин, синаповая кислота, много глюкозидов кверцетина. В проросших зародышах наблюдалось самое высокое содержание гиббереллина. Находится тоже и ингибитор. Проросшие зародыши в пересчёто на сухую массу содержат в 5 раз меньше этого ингибитора, чем зародыши из нестратифицированных семян. В этих зародышах уменьшается количество ауксин, это связано с большим запросом этого вещества в процессе роста.

Покой семян дикой черешни связан с наличием ингибитора. Во время стратификации, с уменьшением покоя, уменьшается отношение содержания ингибитора к содержанию стимуляторов. Уменьшение покоя семян связано с ростом содержания эндогенных стимуляторов роста ауксин и гиббереллин.

Гиббереллин, который появляется в семенных оболочках в стадии трескающих косточек, вероятно принимает участие в регулировании гидратации и активности этих оболочек, а также эндоспермов в процессе растрескивания косточек.

На основании биологических тестов и хроматографичных проб установлено, что доминирующими ауксинами являются, вероятно, IAA. Кроме этих ауксин в зародышах находятся ещё и иные активные вещества в тестах на ауксины, которые на основании растворяемости можно бы определить как кислые и нейтральные.

Доминирующим гиббереллином является, вероятно; GA_3. Кислые и нейтральные гиббереллины не были идентифицированы.

В семенах дикой черешни выступает один ингибитор, который своим поведением и свойствами припоминает абсцисиновую кислоту.

Fot. K. Jakusz

Żywotnik zachodni, odmiana złocista (*Thuja occidentalis 'Aurescens'*)