THE DORMANCY AND GERMINATION OF SEEDS OF THE FRAXINUS EXCELSIOR L.

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Introduction

Dormancy of the *Fraxinus excelsior* i. e. the long-resting period after the ripening of the fruit is commonly known to foresters. Unfavourable experiences made in nursery, insufficient and irregular sprouting of seeds suggested the necessity to examime minutely the problems of this species, economically so extremely important, concerning the dormancy and germination of its fruit.

Data available in the literature are fairly scanty, while the theoretical considerations and practical experiences not infrequently are at variance with each other.

The first analytical work has been made by LAKON (9). He states that the seeds of the *Fraxinus excelsior L.* contain morphologically complete but undeveloped, small embryo. During the stratification the embryo gets its food materials from the endosperm and develops pretty slowly. Germination may occur only in the phase when the embryo had completely filled the space between the two parts of the endosperm. Similar symptoms have been observed in *Fraxinus nigra* by STEINBAUER (14). In his opinion the size of embryo required for germination can be best obtained by storage at 20 C° temperature. Both authors consider the mechanical resistance of the tissues encasing the embryo as primary inhibitory factor of the germination. In MÁTYÁS's opinion (10) the most favourable stratification for the germination of the *Fraxinus excelsior* is that of 3-month duration at 15°—18° C temperature followed for 4—5 months at 0° C. RIMLER (13) thinks early autumn sowing to be suitable. He observed that seeds in the natural stratification are germinating more favourable in spring. According to information from foresters seeds stored early autumn in wet sandy pit at about 5° C for 4—6 months contain fully enlarged embryo and are germinative.

Experimental

I.

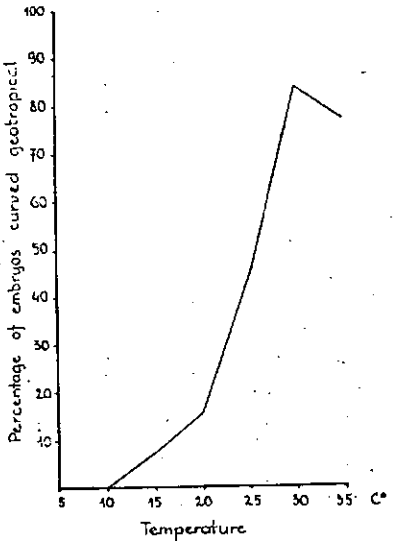
Fruits containing the fully enlarged embryo had passed their dormancy and are germinative. Thus the question arises why is the length of 4—6 months required for the initial development and growth of a few mm.

On the other hand there is the question whether the primary cause of »dormancy« is to be looked for in the embryo or in the enveloping tissues or in the relation of the embryo and of the enveloping tissues.

To settle the problem the embryos of ripe seeds, harvested in November, after 24-hour soaking were carefully excised, placed 100—100 in each Petridish on moist filter paper, put in thermostat ad different temperatures within 2 weeks from harvest. Initiation of the development of the embryos beside the rapid elongation is shown also by the geotropic curvature of the radicula.

Number of developing embryos on the 10th experimental day is shown by graph 1.

On the basis of the data obtained the development of the excised embryos — especially at higher temperatures — begins rapidly.



Graph. 1.: Growth ability of the embryos.

On the other hand there is evidence that the normal germination as well as the initiation of growth within the embryos in the intact seed fail to occur under similar conditions. The embryo within the seed is in a »forced« dormant condition. Consequently inhibitory factors of development are to be looked for not within the embryo but in the relation of the embryo and of the enveloping tissues.

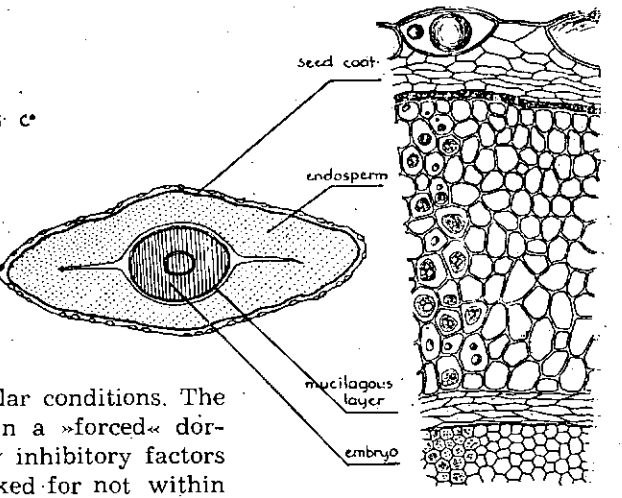


Fig. 1.: Cross section of the seed.

II.

In the opinion of the authors mentioned above dormancy is due to the mechanical properties of the layers enveloping the embryo. As the fruit coat is rather thin easily permeabile by water and oxygen, and rapidly rotting in the soil owing to microorganism activity, role in the mechanical inhibition can be played only by the layers of the seed.

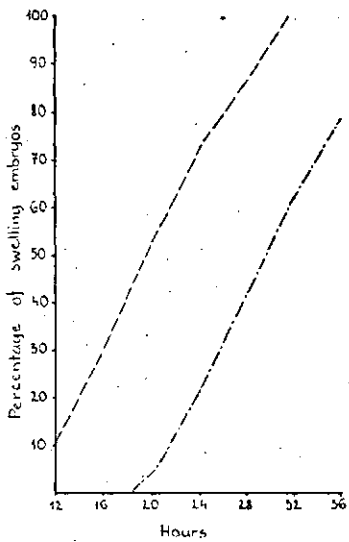
Tissue structure of the seed is shown in Fig. 1.

Due to the structure two layers can be considered as inhibitory causes: the seed coat and the endosperm proper. Presumably these layers may play a role

in blocking the water and air, further as a mechanical resistance may arrest the expansion of the radicula.

To determine whether the afore mentioned layers form a significant inhibitor in the water uptake, 300—300 harvests and seeds without fruitcoat have been soaked in well-water and observed the time the water penetrates into the embryo.

The water uptake required for the germination is precisely shown by the clearly discernible swelling of both the mucilaginous layer enveloping the embryo and the embryo itself. The water saturated condition of the embryos has been microscopically observed in 20—20 cross- sections made hourly. The data are shown in Graph. 2.



Graph. 2.:
Intensity of water uptake.
—·—·—·— seeds with pericarp.
— — — seeds without pericarp.

The observations reveal that the embryo of seed, coated or not, becomes turgescent within 48 hours, meaning that the layers enveloping the embryo are water-permeable.

The fact that the structure of the fruit, thickness of the layers and waterpermeability of the *Fraxinus pennsylvanica Marsh.* well germinative after a short dormant period is similar to that of the *Fraxinus excelsior,* makes doubtful, whether the inhibition is due to its being air-tight.

The layers of the fruit enveloping the embryo exert a retarding effect on the germination of the seed only after the dormancy, i. e. its leaving the seed of the radicula of the fully enlarged embryo. Removing the part toward the radicula of the seed containing the undeveloped embryo no germination occurs, while seed of enlarged embryo after its period of dormancy in humid conditions begins to germinate within a few hours.

The resistance of the enveloping tissues gives no satisfactory explanation to the very slow, sometimes even in favourable conditions, 4—5 month lasting embryo-growth within the fruit.

III.

In view of the results of the above observations the presence of a so far unknown inhibitory factor outside the embryo, but inside the fruit, can rightly be assumed. We suppose the presence of an inner inhibitory substance.

For preliminary information ground seeds were extracted in distilled water (vol. 1:10) at room temperature for an hour, the extract, being filtered, was placed in Petri-dish on double filter paper and thereon test seeds of fresh Sinapis alba were germinated in dark at 26° C. After 24 hours a tremendous difference appeared in the germination; in contrast to the 98% average of the control only 1% of the seeds on the filter paper, containing the extract, germinated.

The next step was to examine, in which layer of the fruit is the inhibitory substance localized. The dry fruit was separated in layers and the seeds of the Sinapis have been left to germinate in 1:10 diluted aqueous extract of the different layers in identical condition as before. Fig. 2. shows the results of germination after 24 hours on the basis of the average of six parallel experiments.

Fig 2. clearly shows that the inhibitory substance can be demonstrated from three layers of the fruit: pericarp, seed coat and the mucilaginous layer clinging to the embryo. The innermost layer is proved to be the most effective. Considerably less effective is the seed coat, while the extract of the pericarp is the least inhibiting. The extract of the endosperm closely amounts to that of the control. In all probability inhibitors of the single layers are not identical.

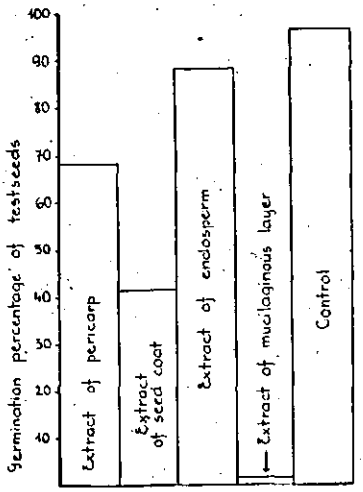


Fig. 2.: Localisation of the inhibitory substances.

The substance of the mucilaginous layer may presumably be accounted for the dormancy, the causative factor of the slow growth of the embryo — due partly to the immediate vicinity, partly either to the highest contents of inhibitory substance or to the greatest intensity of the effect.

In connection with this assumption the following questions were to be cleared up: (1.) as no germination can begin due to the original concentration, does the amount of the substance in the mucilaginous layer decrease and what is the rate of losing its inhibitory effect in the course of its stratification process, (2.) what is the rate of the rapidity of the embryo growth, (3.) if there is any correlation between these two processes and (4.) what relation may be between the mentioned processes and germination of the ash seeds.

In order to give answer to these questions dry fruits harvested in November (Tõs-forest in the County of Bács-Kiskun) have been stratified in river sand of 15 weight % of water-contents for 6 months at 5° and 20° C. Samples have been tested fortnightly.

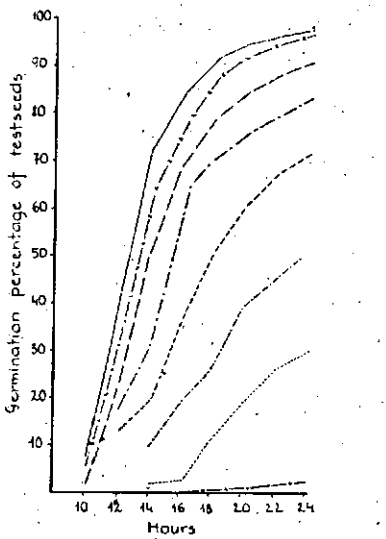
(1.) To examine the decrease of inhibition the substance ot the mucilaginous layer has been diffused on filter paper* in a Petri-dish of 5 cm diameter. The doubled filter paper has been put in the dish and the seeds, being well soaked in distilled water, cut longthwise, were placed thereon close to each other, cut side downward. In this way every dish contained 6 half seeds. The diffusion lasted for 6 hours at 20° C in dark. After this period the ash seeds were removed and Sinapis seeds, used as tests, were placed on the filter paper. The germination has been made at 26° C in dark. The intensity of ger-

* Macherey-Nagel qualitative filter paper No. 637.

mination denotes always precisely the amount of the inhibitory substance. Examination has been carried out every second week in 5—5 parallel courses during the 6-month storage. The germination intensity of the test seeds in different periods an at various temperature i. e. the change of the monthly amount of the inhibitory substance within the ash fruits are shown in Graphs. 3. and 4. Data of the Graphs. represent the means of the parallel examinations.

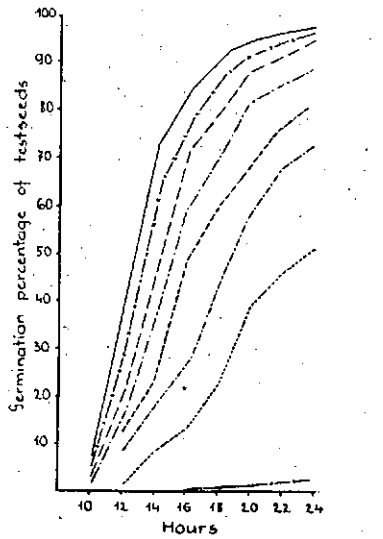
According to the results obtained the concentration of the inhibitory substance in proportional to the duration of the storage and depends on the temperature.

In the initial period of the storage the decomposition of the inhibitor is rapid at both temperatures. The minimal concentration of the substance found in the germinating ash fruits at 20° C is being approached already in the second month. The amount of the inhibitor decreases more rapidly at 20° C



Graph. 3.: Rate of decomposition of the inhibitory substance at 5° C.

—+—+— dry seeds
..... after 1-month stratification
- - - - - - - - - - after 2-month stratification
---------- after 3-month stratification



Graph. 4.: Rate of decomposition of the inhibitory substances at 20° C.

—·—·—·— after 4-month stratification
— — — — — after 5-month stratification
—×—×— germinating ash seeds
———————— control

than at 5° C the curve of the second month, however, shows a relatively lower condentration even in the latter case.

The question is now whether the contents of the substance of the other two inhibitory layers is liable to change i. e. to decrease. The method of this examination is analogous to that described in Chapter III. The effect of the pericarp from this point of view is negligible due partly to the adsorptive property of the soil and partly to the decomposing activity of microorganisms. On the other hand the amount of the inhibitory substances in the coat of the seeds being in germination, is almost identical with that of the freshly harvested ones, does not decompose, consequently it can hardly play a considerable role in

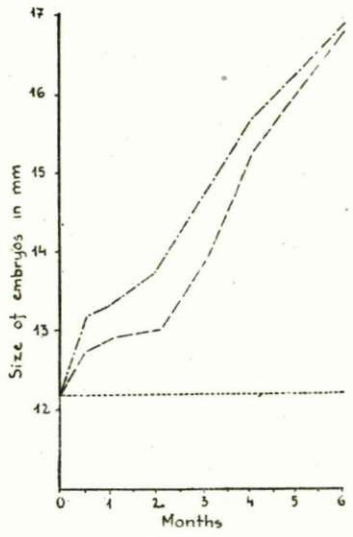
influencing the germination. Thus taking into consideration these results it is evident that the indication of a decrease of the substance in the test seeds alone is showing the change of the substance of the mucilaginous layer playing a decisive role in influencing the germination.

(2.) The growth of embryo has been also fortnightly observed. The pertiment data represent the means of 30—30 measurements. Size of growth of the embryos within the fruits is to be seen in Graph. 5. and Photoes a.—c.

Embryos in humid storage are gradually developing. The size is proportional to the time of the storage and depends on the temperature.

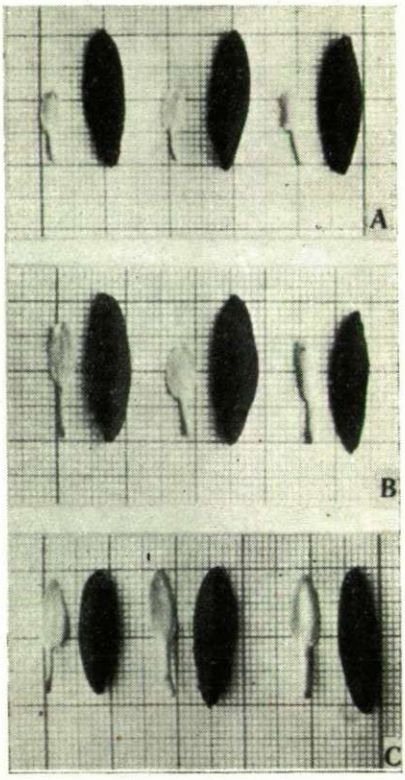
The growth period on the basis of the development of the embryos, may be divided into three parts. In the first phase (2 weeks) the expansion is rapid owing to turgescence observable already on the second or third day. The second phase (till the second month) is the slow growth period while the third is that of accelerating growth. The arrangement of the phases in particularly characteristic of the seeds stratified at 5° C.

(3.) The question is whether there exists a correlation between the decrease of the contents of the inhibitory substances and the embryo growth.



Grap. 5.: Rate of growth of the embryos within the seed stratification.

— — — — — at 5° C.
—·—·—·— at 20° C.
---------- control, dry seeds



Photograph: Relation of size of the seed and of the embryos

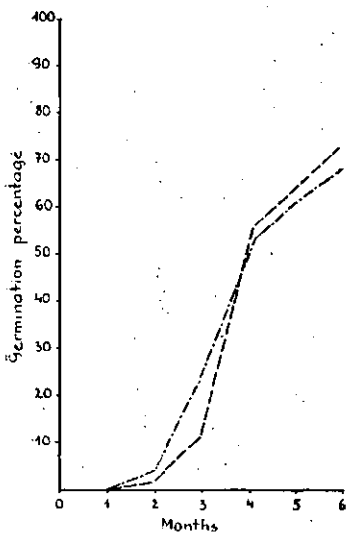
A. when ripened;
B. after 2-month stratification
C. after 6-month stratification.

Comparing the Graphs. (3—4 and 5) may be stated: the smaller the amount of the substance, the larger the embryo. The decrease of the inhibitor is ever followed by an intensive growth of the embryos. In the first month the growth is very slow even in a relatively high concentration of the substances. A considerably decreasing inhibition is manifested in the second month while in the third and the following period an accelerating growth is evident. In the fourth month when hardly any substance can be found in the seeds, the embryos are approaching the optimal size required for the germination.

(4.) At last had to be stated whether the results obtained in this test are confirmed by the intact seeds, i. e. when is the deconposition of the inhibitors to such an extent as to initiate the germination of the ash seeds.

To determine this germination tests on the stored seeds were fortnightly made in washed river sand containing 20% water at a seemingly optimal temperature 10° and 30° C alternating in periods of 12 hours. Tests were carried out with 100—100 seeds in three parallel series. Results up to the 14th day of each test are seen in the Graph. 6.

The data obtained are in accordance with those shown by the Sinapis-test. The average of the germination is as a rule proportional to the ammount of the inhibitors, to the average of the size of the embryo resperctively. With the decreasing concentration of the inhibitory substances and with the embryo growth the percentage of the germination is equally increasing. Particulary characteristic is the rapid increase following the 4th month which may be related to both the then considerably decreased contents of the inhibitors and the accelerated growth of the embryos after the second and third month. The curve of the seeds stored at 5° C in the 4th month is somewhat higher than that of those stratified at 20° C. This indicates that the low temperature has a beneficial effect on the formation of the metabolic process required for the germination.



Graph. 6.:
Rate of germination of ash the seeds after stratification.
— — — — — at 5° C.
—·—·—·— at 20° C.

It is worth mentioning that the data of the above described investigations are supported by other microbiological observations. According to my observations the inhibitory substance of alone the mucilaginous layer is able to inhibit the increase of a series of bacteria-species. This bacteriostatic effect decreases during the stratification, later ceases.

IV.

The laboratory investigations raised by practical problem as a rule by way of application returns to practice again. It may be assumed that the present methods based merely on empirical experciences are fairly satisfactory. On the basis of the data obtained may be concluded that most favourable temperature

for stratification in the first phase is that of room while in the second that of low temperature.

With the knowledge of recent experiences and results we have the possibility of devising quisker and less complicated methods. The first attempts to devise such a new method are fairly promising and encouraging.

Summary

1. The primary cause of the dormancy can not be attributed to the embryo rest. Excised embryos begin to develop in wet condition.

2. Tissues enveloping the embryo mean no serious difficulty to water penetration and play insignificant part in the oxygen retention.

3. Germination inhibitors are found in the pericarp, seed and in the mucilaginous layer.

4. The substance of the mucilagionus layer exhibits the most intensive and immediate inhibition which, however, gradually becomes ineffective.

5. The embryo growth and germination of the ash seeds are closely related to the ammount of the inhibitory substances present in the mucilaginous layer.

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