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DORMANCY IN FRUITS OF TILIA PLATYPHYLLOS SCOP. I

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Summary

A study was made of the dormancy of seeds of *Tilia platyphyllos* SCOP., with special regard to the clarification of the roles of the pericarp, the testa and the endosperm in the maintenance of the seed dormancy. It was found that under moist conditions the seeds require several months at low temperature (5–6 °C) to pass through the after-ripening, which is the condition of germination. The seeds have no need for a photoperiod, light and darkness both being ineffective. Germination is hindered by the presence of the thick pericarp and the seed-coat, which prevent the washing-out of the inhibitors. The inhibitors cannot be removed from the intact seeds by washing, whereas they can be washed out of the bare seeds (without seed-coat) in 24 hours. The excised embryos grow on moist filter paper and on *White* culture medium with a constant intensity, independently of the pre-treatment. The germination is not stimulated by thiourea, KNO_3 or ethylene chlorohydrin, and even the effect of gibberellic acid is very moderate.

Introduction

Tilia platyphyllos SCOP. is widespread throughout the whole of Europe; it mainly forms woods in mountainous regions, but it is also planted. The ssp. pilous on the leaf veins was selected for study. Its unopening, unilocular fruit normally contains one seed (Fig. 1). The fruits are in deep dormancy, and under normal conditions their germination occurs in the second spring following the formation of the fruit. In forestry practice the fruit is stratified into sand in the open (kept at 3–5 °C for about 150–180 days), and then sown in the second spring.

The freshly gathered seeds of a considerable proportion of the woodland trees indigenous to the temperate zone are in dormancy and require an after-ripening period at low temperature (5 °C) to become capable of germination. Attempts have been made to elucidate the basis of the mechanism of dormancy by the detailed study of the biochemical processes occurring during the after-ripening (COLMAN, 1961; FRANKLAND, 1961; PINFIELD, 1965; FRANKLAND and WAREING, 1966), but those changes which can be followed during the after-ripening, such as the increase in activity of the enzymes and the mobilization of the reserve substances, can also be observed on the germination of the seeds without dormancy, and it thus appears that they are not closely connected with the after-ripening.

It seems more significant to study the relation of growth-stimulating hormones and inhibitors in the extracts by chromatography, coupled with appropriate biotests. For example, the decrease of the amount of inhibtors in *Acer* (PHILLIPS and WAREING, 1958) is in correlation with the cessation of the bud dormancy. The inhibitors have a confirmed role in the seed dormancy in *Xanthium* (WAREING and FODA, 1957), *Betula* (BLACK and WAREING, 1959) and *Fraxinus* seeds (VILLIERS and WAREING, 1960; SZALAI, 1965, SZALAI and NAGY, 1968).

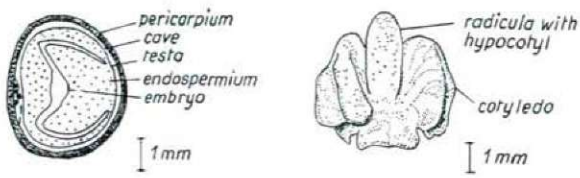

Fig. 1. Cross-section of fruit of *Tilia platyphyllos* Scop. (A) and embryo excised from the endosperm (B).

In contrast to the fairly abundant literature data referring to the dormancy of *Fraxinus, Acer, Corylus* and *Fagus* sp. seeds, no literature data were found for *Tilia platyphyllos* as regards the state of dormancy of the fruits or seeds.

An account is given below of our experimental results, the aim of which was to establish:

1. how fruits and seeds imbibed with water behave under normal germination conditions in the period following the ripening of the fruit;

2. whether there is a difference in the behaviour of fruits and seeds subjected to low temperature;

3. whether the embryo begins to grow in the imbided seeds;

4. the effect of washing out the fruits and seeds with water;

5. the germination-stimulating effects of various dormancy-terminating agents.

Materials and Methods

The study material was obtained from the Csongrád County Forestry Station; it originated from trees forming a closed plot, and thus population material was studied. Petri dishes, washed sand and Schleicher—Schüll filter paper were used for the germination tests, with boiled water for the moistening. The fruits and seeds were preliminarily sterilized with 0.1% sublimate solution.

Variations:

1. Germination:
   a. in constant light (6000 lux, at 6 °C and 25 °C);
   b. in constant darkness (at 6 °C and 24 °C);
   c. in 12-hour alternating periods of light and darkness (at 24 °C).
2. Seeds germinated under the conditions given in (1) were prepared at 25-day intervals, and the development of the embryo was examined.
3. The stratification was performed in a refrigerator at 4—5 °C.
4. The fruits were soaked in distilled water and mildly shaken for 48 hours, and then subjected to germination as under (1).
5. After scarification the fruits were treated with thiourea, ethylene chlorohydrin, potassium nitrate and gibberellin.

Results

1. Germination tests

Immediately after the falling of the fruit, the fruits and seeds were sown in washed sand in Petri dishes, but to facilitate the imbibing they were treated with concentrated sulphuric acid. Since sulphuric acid treatment involves the danger of the acid penetrating the hilum and destroying the tissues of the seed, it was considered advisable to investigate the connection between the various times of scarification and the extent of damage. On this basis a „pickling" time of 15 minutes appeared to be the most effective. The relation between the time of sulphuric acid treatment and the swelling is given in Fig. 2.

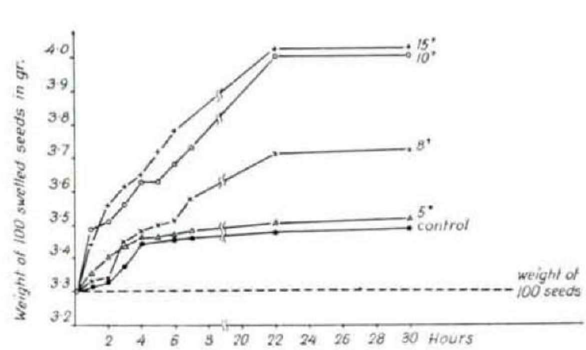

Fig. 2. Relation of the duration of the sulphuric acid scarification and the time necessary for the swelling of the seeds.

Efforts to induce germination immediately following the falling of the fruit did not lead to success, for there was practically no germination during 120 days in constant light (at 25 °C), in constant darkness (at 24 °C), or in alterating light and darkness (at 24 °C); only a few seeds burst open (7%, maximum), but the radicle did not emerge. Under similar conditions but at 6 °C bursting of the seeds occurred in a higher percentage (> 50%), but even then the radicle did not appear.

2. Development of the embryos in the swollen seeds

When the seeds were opened it was found that under the most varied germination conditions (1a, 1b, 1c), both in the cold and at laboratory temperature, the embryos remained in dormancy (Fig. 3). On the other hand, if the excised embryos were placed into *White* nutritive solution or moist filter paper in Petri dishes, they began to grown. From a graph showing the growth on the *White* medium (Fig. 4) it can be seen that the embryos grew here with an approximately constant intensity, regardless of the nature of the pretreatment; this indicates that the growth of the embryo is inhibited by the endosperm or the seed-coat, or by their joint presence.

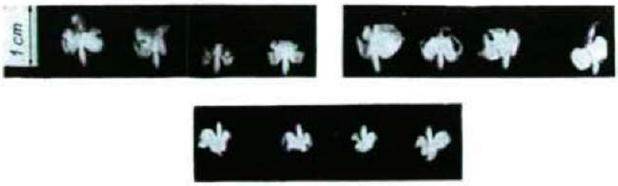

Fig. 3. The development of embryos excised from *Tilia* seeds after various pre-treatments. From left to right in pairs: in constant light at 6 °C and 24 °C, in constant darkness at 6 °C and 24 °C, and in 12-hour light and dark periods at 24 °C.

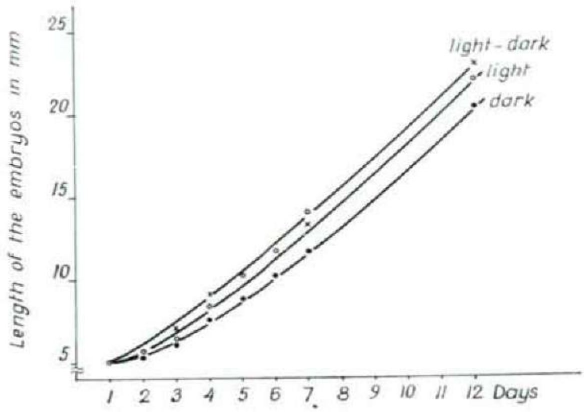

Fig. 4. Development on *White* medium of excised *Tilia* embryos after various pre-treatments, on the 1st—12th days following the excision.

3. Stratification

For many species the imbided seeds can be aroused from their state of dormancy by subjection to low temperature. The stratification of the *Tilia* seeds paper wadding, and in the other case in moist sand for 3 months at 6 °C, and the germination was then followed at 20 °C. It was found that 65% of the seeds stratified in the sand germinated in the fourth month of the treatment, whereas only 60% of those in the paper wadding germinated and only in the fifth-sixth months. The proportion and vigour of germination are shown in Fig. 5.

4. Effect of washing out seeds and the embryo

It was assumed that the inhibitors maintaining the dormancy of the seeds are water-soluble, and accordingly can be removed from the seeds by washing. The scarified seeds were washed for 24 hours under running water, and then for a further 24 hours in water changed every 2 hours, with gentle shaking. This was followed by germination according to (1).

5. Effects of various dormancy-breaking methods

The fruits were scarified, then treated with thiourea, ethylene chlorohydrin, KNO_3 or gibberellin to stimulate the germination, and finally placed in a moist environment. All these substances have been employed with good results to terminate the dormancy in various species. With the exception of gibberellin, none of the techniques used led to germination in the present case; the cause of this considered to be that the substances did not penetrate into the seeds to a sufficient extent. Only in the case of gibberellin was a slight stimulating effect observed, when the gibberellin treatment was followed by germination at low temperature. Gibberellin was used to stimulate the scarified seeds in various concentrations, with the results shown in the Table.

It can be concluded from the results that GA_3 in itself can not be regarded as the initiating factor, for its effect, which was moderate, appeared only in the chilled seeds.

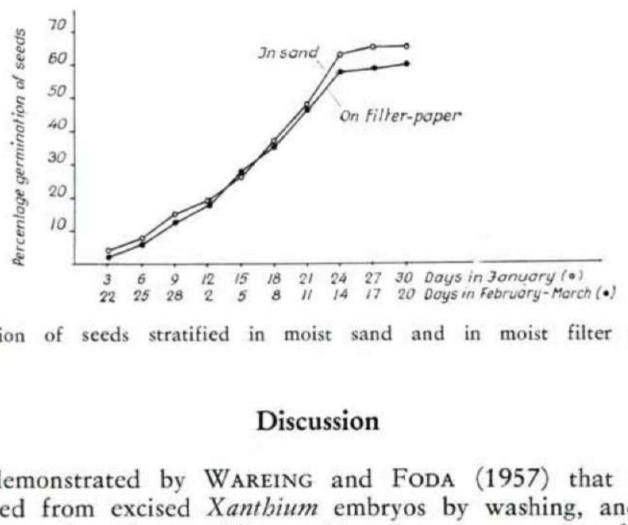

Fig. 5. Germination of seeds stratified in moist sand and in moist filter paper at 20 °C.

Discussion

It was demonstrated by WAREING and FODA (1957) that the inhibitors can be removed from excised *Xanthium* embryos by washing, and such ebryos germinate on a moist substrate. The washing-out was not successful in the case of the intact *Tilia* seeds, whereas according to STEINBAUER (1937) and FERENCZY (1955) the inhibitors can be removed from *Fraxinus* fruits even in the presence of the seed-coat. As regards practice, only this latter case would be of importance. Since the dormancy of the intact *Tilia* seeds can not be terminated by washing-out, it must be assumed that the washing-out of the inhibitors is hindered by the seed-coat, or the inhibitor is not water-soluble. In the latter case the destruction of the inhibitors under natural conditions leads to germination. Since the scarified seeds swell relatively quickly, the water-permeable seed-coat freely permits the passage of oxygen too.

It is worthy of note that the various stimulating agents (ethylene chlorohydrin, KNO_3, thiourea) have no effect, a slight stimulation being induced only by GA_3 treatment. These facts indicate that the primary factor in the dor-

Illumination	Constant light 6000 lux						Constant darkness						Alternating light and darkness						Constant darkness					
Temperature	25 °C						25 °C						25 °C						5 °C					
GA_3 concn.	1000	500	200	100	50	control	1000	500	200	100	50	control	1000	500	200	100	50	control	1000	500	200	100	50	control
1. II., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10. II., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
20. II., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
1. III., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10. III., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	1	3	2	—
20. III., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	7	7	4	4	—
1. IV., 1972	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	6	9	11	12	5

mancy of the *Tilia* seeds is the presence of the inhibitors; their remaining in the seed is enhanced by the thick pericarp and the impermeable seed-coat. The first step towards the understanding of the nature of the dormancy must be the analysis of the inhibitor contents of the individual seed-parts.

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