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Content of plant growth regulators in the developing seeds of oak (*Quercus robur* L.)*

II. Auxin-like substances

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INTRODUCTION

It has been proved in a series of investigations that the seeds of many species of plants represent a rich source of auxins (Hatscher 1945; Söding 1952; Audus 1959 and others). Many authors ascribe the essential role in the development of seeds to these compounds (Luckwill 1948; Ruhland 1961; Crane 1964 and others). Luckwill has shown that immature seeds contain particularly high amounts of auxins, while there is often a lack of these substances in the mature seeds. The quantitative changes of these compounds in the course of seeds development may be responsible for their suitable growth and formation. The majority of the former works devoted to the function of auxins in seed development concerned herbaceous plants (Muir 1942; Nitsch 1952; Crocker et al. 1957; Michalski 1960). Only few publications have dealt with the problem of auxins and their role in the seeds of the tree plants. The subject of Luckwill's investigations (1948, 1956, 1957, 1959) was to examine the content of growth regulators in the endosperm of the developing apple-tree seeds. Mimault (1956), in the chromatographical assays on pear and cherry seeds at various stages of their development, has found the presence of auxins and the absence of inhibitors in immature seeds.

Especially little is known about the dynamics of auxins and their role in the seeds of forest trees. Daletskaya (1964), using the chromatographic method, detected considerable quantities of 3-indoleacetic acid in the seeds of maple and spindle tree. In the investigations on conifers, Krugman (1965) identified auxins in immature seeds of *Pinus*, while Michniewicz and Kopcewicz (1968) recorded them in germinating seeds of the same plant. These, not numerous assays, do not allow to draw more general conclusions as to the content and the role of auxins in the seed development of forest trees. There is also a lack of data concerning auxins in the seeds of *Quercus*. It seems thus purposeful to carry on researches of growth regulators in the seeds of this plant.

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METHODS

Similarly as in the investigations concerning the contents of gibberellin-like substances, the oak-tree seeds, (*Quercus robur* L.) have been analysed in seven stages of their development, at three weeks interwals (Michalski 1968). The samples taken for the analysis were following: 25 g for the initial stages of the development (I and II), 50 g for III—V stage, and 100 g samples for VI—VII stage.

In experiments concerning the mode of distribution of auxins in the seed, 100 immature, (12 weeks old acorns) and 100 mature acorns have been divided into embryos, cotyledons and shells.

Extraction and purification: The auxins have been extracted from frozen and homogenized material by means of cooled methanol and have been fractioned according to Larsen's method (1955). Methanol has been used for the extraction in a strict proportion to the weight of the material (4:1 v/v.).

Chromatography: For the bioassays, the auxins contained in the two acidic fractions have been partitioned on Whatman's paper No 3 in solvent isopropanol-ammonia-water (10:1:1 v/v.).

For a closer characteristic of these auxins the rechromatography by means of the TLC-method has been applied on the silica gel G in the solvents:

isopropanol-ammonia-water (85:5:15 v/v.) (Stahl 1962).

chloroform-acetic acid (95:5 v/v.) (Stahl 1962).

chloroform-ethanol (65:35 v/v.) (Ballin 1964).

As developers for indole compounds, the Ehrlich's (E) and Prochazka's (P) reagents have been used. For the intensification of spots, the plates were treated with vapors of aqua regia (Stahl 1962).

Bioassay: The growth activity of auxins, partitioned by paper chromatography has been examined by means of the standard *Avena* section straight growth test according to Bonner on Victory oats-Svalöf. The identified stimulating zones of chromatograms have been additionally controlled by means of the *Agrostemma* hypocotyl section test according to Borriss (1955). The test results have been evaluated statistically.

RESULTS AND DISCUSSION

The results of experiments revealed in the examined plant material the prevalence of two active growth promoters, (R_F 0.0—0.2 and 0.4—0.6) designated as A and B respectively, and two growth inhibitors, (R_F 0.2—0.4 and 0.7—0.9). The typical position in chromatograms of these active substances, obteined from extract of 12 weeks old seeds, in which there could be distinguished the cupules and the acorn, was shown in histogram (Fig. 1).

The quantitative changes of the growth stimulators during the development of oak seeds are illustrated in Fig. 2. The results of analysis of the inhibitors will be the subject of a separate publication.

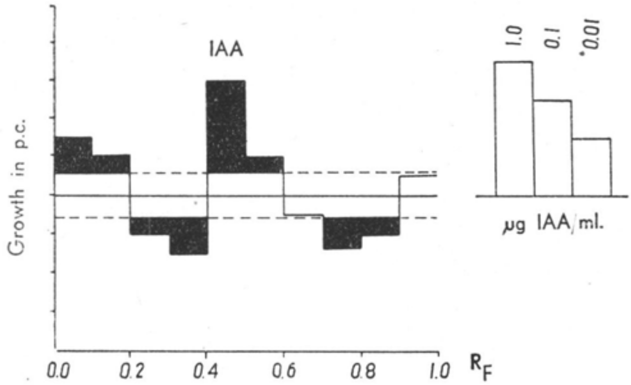

Fig. 1. Bioautography of growth regulators of 12 weeks old acorn of *Quercus robur* L. partitioned on paper

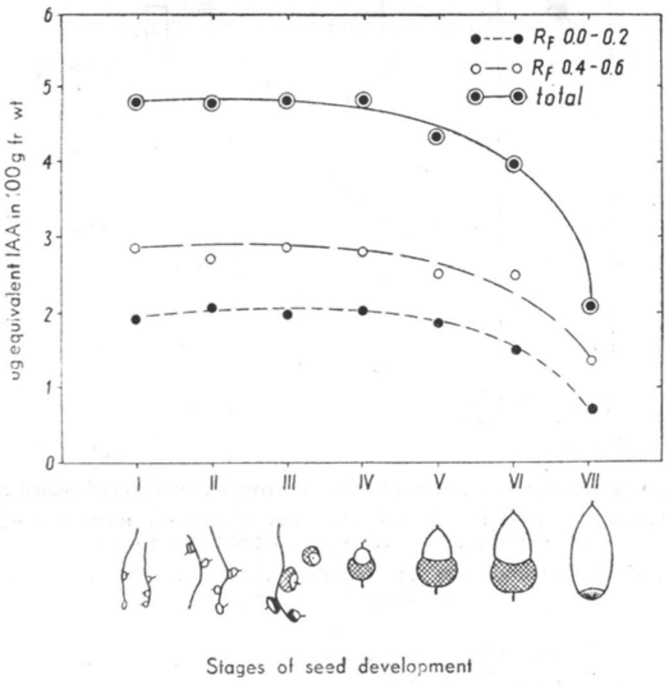

Fig. 2. The dynamics of auxins during oak seed development. (fr. wt. contains 36 p.c. water)

Stimulator (A) localized in the given chromatographic conditions at R_F 0.0—0.2 showed a fairly important growth activity. Its level slightly increased in early stages of the acorn development (stages I—III) but in the course of maturing of seeds markedly decreased.

A similar tendency to level lowering during the development of fruit was also shown by the second active growth substance (B), at R_F 0.4—0.6. Its stimulating effect is considerably higher as compared with the former substance. It occurs in all the stages of the acorn development. The results of rechromatography of the isolated substances A and B on the silica gel plates and the colour reactions with Ehrlich (E) and Prochazka (P) reagents, have shown, a considerable similarity of the stimulator B (R_F 0.4—0.6), with the indole compound (Fig. 3). Also, further

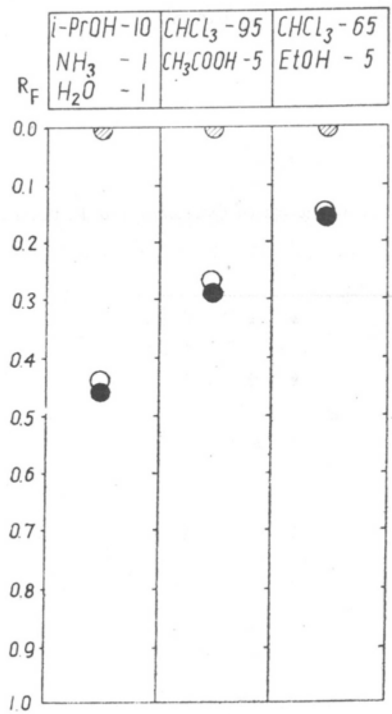

Fig. 3

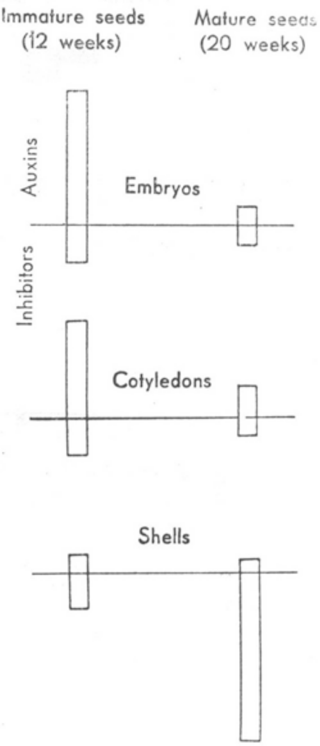

Fig. 4

Fig. 3. TL-rechromatography and colour reaction of growth stimulators isolated from oak seeds black circles — IAA, (E)hrlich — violet, (P)rochazka — yellow; hatched circles — A substance, E — grey, P — orange-brown; white circles — B substance, E — bluish-pink, P — yellow

Fig. 4. The level of growth stimulators and growth inhibitors in the various parts of acorn, as depending on the age of seeds.

comparative studies on the chromatographic properties (R_F value) of this substance and of the authentic sample of IAA in various solvents have been performed. It could be shown that the active substance at R_F 0.4—0.6 was probably 3-indoleacetic acid. The R_F value of IAA in the applied chromatographic conditions was almost identical. The observed displacement of the spots of the natural stimulators on the chromatograms, may be probably caused by the fact that IAA often occurs in a complex form. The colour reaction of the spots with the applied reagents, as well as their fluorescence in the filtered UV would show the identity of the stimulator B with 3-indoleacetic acid. The identification of the stimulator A (R_F 0.0—0.2) was not sucessful. It seems to be similar to 3-indolepyruvic acid. However, the low stability of IPyA in the solvent containing ammonia as well as the acids, presents great difficulties for the identification of this compound, and does not give univocal results.

The analysis of auxins in the acorn shows a great quantitive differences of these substances, depending of the examined part of the acorn (Fig. 4). Particularly rich sources of auxins are the developing embryos of the immature seeds, as well as the cotyledons. The mature seeds do not contain many auxins but considerable quantities of inhibitors, which were accumulated mainly in the seed shells.

The results of these studies are in concordance with the observations of many authors. The investigations of Luckwill (1957); Hashizuma (1965) and many others concerning the dynamics of growth regulators in the developing seeds, have also shown the occurence of various active substances. Particularly many of these substances were contained in immature seeds (Crocker et al. 1957; Niethammer et al. 1961). However, the quantity of auxins decreases rapidly in the course of their maturing (Söding). This fact has been also confirmed by the data of the presented work. Probably these compounds are transformed into a non-active form, being a precursor of auxin. In Luckwill's opinion (1948) the presence of auxins is a fundamental condition of the regular growth and development of the entire fruits. According to this author, the apple-tree seeds, contained at least four different auxins which had the character of acids and neutral substances and which are responsible for seeds maturation. The identification of IAA in the developing seeds of *Quercus* confirms this supposition. There are various opinions concerning the distribution and accumulation of growth substances in the developing seed. Krugman has shown the occurence both of IAA and 3-indoleacetonitrile (IAN) in the developing seeds of *Pinus lambertiana*. In the embryo before the fertilization, there occured mainly IAN, but after fertilization the amount of IAN decreased while the level of IAA increased. The great physiological activity of the embryo distinguishes it among the other parts of the developing seed. It is evident that this may be connected with the high auxin concentration in the developing embryo. In the mature embryo only a small amount of auxin was found. Their highest level has been observed in the earlier stages of the seed development (Hatscher 1945; Guttenberg et al. 1947). In the course of seed maturation its level usually decreased. This was also recorded in the present investigations. In the maturing seeds, the auxin-like substances disappeared and the amount of inhibitors increased. The main place of their accumulation is not the embryo but other tissues, mainly the seed shells. Similar observations are also reported by Grzesiuk (1967). In some plants the growth substances accumulate in the endosperm or cotyledons (Mukharjee et al. 1966). The last observation could not be confirmed in our experiments with oak.

CONCLUSION

1. Two auxin-like substances, of a high activity have been isolated from the methanol extract of the seeds of *Quercus robur* L. analyzed is seven various stages of development. Beside them there occurred also two growth inhibiting substances.

2. It has been shown that with the progress of seed development, the level of the both stimulators markedly decreased. Its lowest amount was found in the matured seeds.

3. One of the stimulators was identified as 3-indoleacetic acid. It occurs in all the stages of seed development.

4. The highest level of auxin was found in the embryos of immature seeds, while the growth inhibitors were found mainly in the shells of the matured seeds.

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Zawartość regulatorów wzrostu roślin w rozwijających się nasionach dębu (Quercus robur L.)

II. Substancje auksynopodobne

Streszczenie

Z ekstraktu metanolowego z nasion *Quercus robur* L., analizowanych w 7 fazach rozwojowych, wyodrębniono dwie aktywne substancje o charakterze auksyn. Obok nich występowały dwie substancje hamujące wzrost. Stwierdzono, że w czasie rozwoju nasion, poziom stymulatorów ulegał stałemu obliżaniu. W nasionach dojrzałych był on najniższy. Jeden z stymulatorów zidentyfikowano jako kwas 3-indolilooctowy. Występował on we wszystkich fazach rozwoju nasion. Najwyższy poziom substancji auksynopodobnych stwierdzono w zarodkach niedojrzałych nasion. Duże ilości inhibitorów znaleziono w łuskach nasion w pełni dojrzałych.