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

I. Gibberellin-like substances

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INTRODUCTION

Much attention has been already given to the problem of gibberellin-like substances in seeds. Remarkable amounts of them have been found especially in immature seeds as for example in the seeds of *Phaseolus multiflorus* (Radley 1958), *Lupinus luteus* (Imamura et al. 1960), *Pisum sativum* (West et al. 1956). It has been shown that the amount of gibberellins was considerably diminished in fully matured seeds. It is considered that gibberellins take part in the processes of seed formation, since a clear correlation has been found between the content of gibberellin and the size of the seeds (Corcoran and Phinney 1962).

Investigations concerning the changes of gibberellin-like substances have been performed mainly on some herbaceous plants (Murakami 1959, 1961, Corcoran and Phinney 1962, Rejowski 1964, Maheshwari and Johri 1965, and Stoddart 1965).

However, very little is known about the dynamics of these substances in the developing seeds of trees. The only known publication concerning this matter is that of Kentzer's (1966) who experimented with ash seeds.

METHODS

Seeds of the oak (*Quercus robur* L.) were analysed at seven phases of development, i.e. since the moment of fertilization till maturation. The experiments started in the 3rd decade of May 1966 were carried out till the end of September at 3 weeks intervals.

At the beginning of the experiments (I—II phase) 25 g samples of seeds were taken for each analysis. In the later phases (III—V), as the seeds developed, 50 g

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samples of seeds were used and in the final phases (VI—VII), 100 g samples of seeds were analysed.

Extraction and purification: The extraction of gibberellin-like substances was carried out in 70% acetone. The plant material was previously frozen at -20°C and subsequently homogenized.

The preliminary purification of extracts was performed by applying two-dimensional chromatography with water-water as solvent (Mowat 1963). This procedure assured the removal of growth inhibitors and also some impurities. The active zone containing gibberellins was eluted with acetone, evaporated to a small volume and rechromatographed by the TLC method.

TLC-chromatography: The gibberellin-like substances were examined by TLC methods on silica gel G and by applying various solvents. The silica plates were prepared according to the classical method of Stahl (1963) and developed in the following solvents:

water,

benzene — acetic acid (10:3 v/v),

benzene — n-butanol — acetic acid (70:25:5 v/v).

The chemical identification of gibberellin-like substances was based on the reaction with conc. H_2SO_4 and on the characteristic fluorescence in UV.

Bioassay: The growth activity of the substances examined was estimated in relation to various test plants —

Lettuce hypocotyl (cult. Böttner) after Frankland and Wareing (1960),

Oat first-leaf (Victory oats — Svalöf) after Michniewicz (1961),

Dwarf pea stem (cult. Kelvedon Wonder) after McComb and Carr (1958).

All analyses were three times repeated whereby each variant was bioassayed in relation to ten test plants. The results were evaluated statistically.

RESULTS AND DISCUSSION

From among the solvent systems applied, the most suitable for partition of gibberellin-like substances was benzene-acetic acid. In this case the active substances were divided into three different groups with R_F 0.0—0.1, 0.5—0.7 and 0.9—1.0, respectively, (Fig. 1).

The chromatogram section between R_F 0.0—0.1 included a substance which reached its maximum level at the IV and VI development phase of fruits. At that time the immature fruits were already full-sized and also clearly differentiated into cupules and seeds (Fig. 2). As known from literature (Sembdner et al. 1962) and own comparative studies, in the above mentioned zone of the chromatogram there appeared usually gibberellic acid (A_3) and GA_1 .

The highest level of gibberellin-like substances localized at R_F 0.5—0.7 was observed in the IV—VI phase of fruit development when they were already fully

grown in size, but remained still immature. With the advancing maturity of seeds, the level of the active substances localized in this zone of the chromatogram slowly decreased. A closer characteristic of these substances revealed, that they were similar to gibberellins A_4 and A_7 .

Fig. 1. Bioautography of gibberellin-like substances from immature seeds of *Quercus robur* L. (phase V), partitioned by the TLC method on silica gel G in benzene-acetic acid (10:3 v/v) as solvent.

This similarity was based on the chromatographic properties of these substances in benzene-acetic acid (R_F 0.60 and R_F 0.57) as well as on their physiological activity in various biological tests.

The third active compound (R_F 0.9–1.0) was also a gibberellin-like substance but of a weaker physiological activity. It reached the maximum level at the III–V phase of seed development, at the beginning of the differentiation of fruits into cupule and seed. From that time on the activity of this substance remained generally unchanged until full maturity of the seeds. The R_F value of this substance corresponded to that of gibberellin A_9 (R_F 0.90).

The distinct character of the particular gibberellin-like substances obtained

from the developing oak seeds has been confirmed by the different physiological activities of these substances in relation to the following test material: the first leaf of oats, the lettuce hypocotyl and the stem of dwarf pea.

In accordance with the data from literature, the relationship between growth activity of certain gibberellins, with regard to the first leaf of oats, is as follows:

$$A_3 > A_1 = A_4 = A_7 = A_9 \text{ (Michniewicz 1963)}$$

whereas in relation to the lettuce hypocotyl test the activity of the same gibberellins was as follows:

$$A_7 > A_3 > A_4 > A_9 > A_1 \text{ (Brian et al. 1964).}$$

The following activity of various gibberellins

$A_3 > A_1 = A_7 > A_4 > A_9$ (inactive) (Brian et al. 1964) in relation to the dwarf-pea, clearly distinguishes these plants from the other biological tests.

Fig. 2. Activity of gibberellin-like substances during oak seed development (fr. wt. contains 36 p.c. water)

Considering the total level of all gibberellin-like substances separated from oak acorns it may be stated that they showed essential changes during the development of seeds. Unripe, but fully developed seeds, contained considerable amounts of these substances. The level of gibberellin-like substances decreased progressively with the maturing of seeds. This fact has been confirmed also in relation to the seeds of some other species as for instance by Kentzer (1966) in the developing seeds of ash.

The varying level of gibberellin-like substances at the particular developing

stages of seeds suggests, that their development is closely related to the activity of these substances. This opinion is confirmed by experiments with gibberellin exogenously applied on the ovary. It was found that under the influence of gibberellic acid there embryoless seeds were obtained (Grzesiuk 1967), whereas the activity of 3-indoleacetic acid caused the development of seedless fruits. This is a quite important point in showing the physiological role of gibberellin in the development of seeds.

The part played by endogenous gibberellins should not be limited to the process of seed formation only. As it has been shown they also were an important factor regulating the resting period of seeds and also their sprouting (Villiers et al. 1960, 1963, 1965).

CONCLUSIONS

1. Oak seeds, analysed in seven phases of development, contained three different groups of gibberellin-like substances.
2. It was found that during the development of seeds, the level of gibberellin-like substances first increased and then gradually decreased with the progress of maturation.
3. The highest level of gibberellin-like substances is observed in unripe but morphologically developed seeds.
4. The essential changes of gibberellin-like substances at the particular developing stages of seeds suggest, that their development is closely related to the activity of these substances.

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

I. Substancje giberelinopodobne

Streszczenie

Metodami ekstrakcji, frakcjonowania, chromatografii i testów biologicznych badano substancje giberelinopodobne w rozwijających się nasionach dębu. W 7 fazach rozwojowych z nasion wydzielono trzy grupy substancji giberelinopodobnych. Stwierdzono zmiany w poziomie tych substancji w zależności od fazy rozwoju nasion. Najwyższy poziom substancji giberelinopodobnych cechowały nasiona niedojrzałe lecz w pełni morfologicznie wykształcone. W nasionach dojrzałych stwierdzono znacznie niższy poziom tych substancji. Różnice w poziomie substancji giberelinopodobnych w nasionach rozwijających się wskazują, że rozwój ten jest związany z działaniem tych substancji.