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The Role of Endogenous Growth Regulators in the Differentiation Processes of Walnut (*Juglans regia* L.)

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Abstract. The character of endogenous growth substances was investigated in developing buds, young fruits and mature walnut leaves. The relatively high content of auxins and gibberellin-like substances was found by means of bioassays in the youngest primordia of vegetative buds. The level of auxins drops in the further course of primordia transformation into the staminate catkins. The development of leaf-buds is characterized by the accumulation of inhibitory activity as revealed by the *Avena* bioassay, whereas the data obtained from the lettuce bioassay indicate a pronounced stimulation. The onset of terminal bud development is also accompanied by inhibitions and it is only with pistillate flower differentiation that the temporary rise in auxin level is observed. An inhibitory activity was found in these extracts using lettuce bioassay. There is a relatively high auxin level in young fruits, mature leaves and resting buds during the midsummer period whereas the accumulation of clearcut inhibitions is signalled by the results of lettuce bioassay.

The regulatory role of growth substances in differentiation may be better understood during the second year as many leaf-abnormities appear only with the outgrowing of the bud. Abnormal catkins differ in the number of florets and stamens and some even bear pistillate flowers. Fruit development is liable to deviations in the early stages of differentiation. Abnormal fruits enable us to elucidate many structural peculiarities.

The young meristems of walnut buds may develop in three different ways as stamen primordia, as vegetative buds or as terminal buds with pistillate flowers. According to ATSMON and GALUN (1962) the pattern of primordia development is regulated hormonally with a strong modifying influence exerted by the adjacent leaf. HESLOP—HARRISON (1964) stresses the importance of substances of auxin character in the sex differentiation and in the general development of inflorescence. The investigation of endogenous growth substance levels in young meristems during primordia differentiation helps us to understand the different patterns of their development and provides the means for selecting the proper kinds of synthetic regulators to influence developmental processes (SLADKÝ 1969).

In our previous work (LANGROVÁ and SLADKÝ 1971) we came to the conclusion that different kinds of walnut buds differ in auxin, gibberellin and inhibitor content and that their course of the development may be altered by the exogenous application of growth substances. The character of endogenous regulators during the development of staminate-, leaf- and terminal. buds has been followed in detail in the present paper. We have also tried to form a true picture of the accumulation of growth regulators in young fruits, mature leaves and buds during the summer season. Modified bud development has enabled us to understand more clearly the role played by regulators in guiding the general course of the processes of differentiation.

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Material and Methods

The experimental material was obtained from a forty-year old walnut growing in the Botanical Garden of the Brno University. The development of the different kinds of buds was followed during the period 1968—1970. Samples were taken representing the onset, middle phase and final stages of bud differentiation. The estimation of growth substances was carried out in the same way as in our previous work (LANGROVÁ and SLADKÝ 1971). The methanolic extract from 15 buds was separated chromatographically using Whatman no. 1 paper. The auxin activity was tested by means of oat coleoptile segment bioassay, whereas the lettuce hypocotyl bioassay was used for the assessment of gibberellin activity. The data from the bioassays are expressed in histograms, each representing the mean value of five buds in a triplicate arrangement. The bioassay variability was estimated as high as $\pm$ 12%.

The character of endogenous regulators in young fruits, mature leaves, catkin buds, terminal and leaf buds was investigated at the onset of the dormancy period. The activity of some standard substances in concentrations 1 and 0.1 p.p.m. (IAA-Lachema ČSSR, GA_3-Phylaxia, Hungary, ABA-Hoffmann La Roche, Switzerland) is indicated on the ordinate to enable us to obtain the quantitative comparison of the presented data.

The morphological analyses were performed to evaluate the abnormities of leaves, fruits and inflorescences. In some cases material was photographed.

Results

The Relation Between Endogenous Growth Substances and Bud Development

The results of auxin and gibberellin bioassays are summarized in Plate 1. The two upper rows of histograms represent the level of auxins and gibberellins in the staminate catkins primordia in the course of differentiation (from 2May to 30May). Auxin activity and gibberellin-like activity occurs in the youngest vegetative primordia. The level of auxins declines in the further course of differentiation and inhibitory substances appear. The final steps of staminate catkin differentiation (30May) are characterized by a notable rise of inhibitions. Gibberellin-like activity is present in the youngest bud primordia and increases further in the following period. It is only at the end of catkin-bud development that the inhibitions start asserting themselves.

The two middle rows of histograms represent the results of auxin and gibberellin bioassays during the leaf-bud development in the period from 16 May to 17 June. These investigations indicate that the formation and differentiation of the vegetative buds is accompanied from the very beginning by a low level of auxin activity as well as by the accumulation of inhibitions. The occurrence of gibberellin-like substances is more pronounced during the early phases of development, the final steps being characterized by inhibitions.

The two lower rows of the histograms represent data on auxin and gibberellin content as related to the development of terminal buds in the period from 9 July to 8 August. The terminal buds originate at the end of the vegetation period when the inhibitory activity prevails. The differentiation of the pistillate flowers is accompanied by an increase in auxins (25 July) whereas the inhibitions appear during the final stages. Gibberellin-like substances were found in the young terminal bud as well as in the final phases of bud differentiation. This very differentiation of the pistillate primordia is, as a rule, accompanied by clear-cut inhibition.

The results of the two bioassays reveal the dynamics of the character of endogenous growth substances in the course of development of staminate-, leaf- and terminal- buds. The occurrence of inhibitions was to a certain extent also ascertained. The accumulation of inhibitory activities stops the growth and differentiation processes and brings about bud dormancy.

We attempted to establish the onset of inhibitions in young fruits, matured leaves, catkin buds and terminal and leaf buds in the year 1970 (Plate 2). The analyses of the extracts (15 June) revealed the early phase characterized by stimulations according to auxin bioassay and by marked inhibitions, according to gibberellin bioassay. It is worth noting that the characters of the histograms for different objects seem to be very much alike in both bioassays. As the leaves consist mostly of active meristems we may assume that the character of growth substances in leaves also affects their level in other organ-primordia. The inhibitions are, as a rule, located at a lower Rf value whereas the stimulations can be found at a higher Rf value.

The Study of Alternative Developmental Potentalities of Buds

Considering the character of growth substances in different stages of bud development a proper kind of regulator may be used to change the pattern of primordia differentiation.

Abbreviations used: ABA-abscissic acid, MH-maleic hydrazide

a. The Reversion of Staminate Flower Primordia into Vegetative Buds

The data given in our previous paper clearly show that spraying with 0,2% IAA or 0.1% MH in the period prior to 1 May may reduce the number of staminate buds and increase the number of vegetative buds. The vegetative buds in the next year gives rise to stems with abnormal leaves. These stems bear leaves with different degrees of lamina development, including even simple leaves (Fig. 1). Similar abnormities resulted from defoliation or appear after damage to the young stems. The primordia which are not yet differentiated start to outgrow immediately following the treatment. Abnormities of a different quality appear, depending on the developmental stage and the treatment used. Similar phenomena occur under natural conditions with a tree damaged by spring frost.

b. The Transformation of Vegetative Buds Primordia Into Staminate Catkins

The spraying of the youngest leaves and branches with 0,3% triiodobenzoic acid on 1 May caused the drop in the number of buds, however, the number of staminate catkins on the stem was increased. The number of stamens and florets within these catkins varied widely in the following year. The application of MH resulted, in some cases, in the abortion of staminate flowers on the spindle of the catkin, as well as in the reduction of the number of stamens in the floret and of the number of florets in the catkin (Fig. 2).

Plate 1. Character of endogenous regulators as revealed by the bioassays in walnut buds during development. The two upper rows represent the auxin and gibberellin levels in staminate catkins. The middle rows represent the regulators in the leaf buds and the lower two rows in the terminal ones. The values for IAA and ABA in an auxin coleoptile bioassay (1 and 0.1 p.p.m.) and for GA_3 and ABA (below) in a lettuce bioassay are indicated in the middle row for comparison. ◀

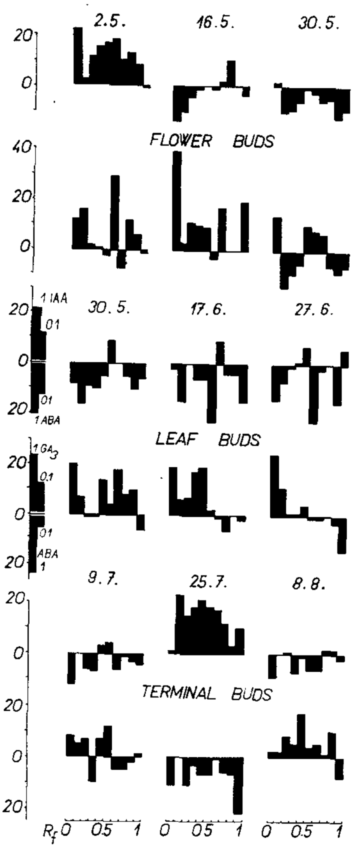
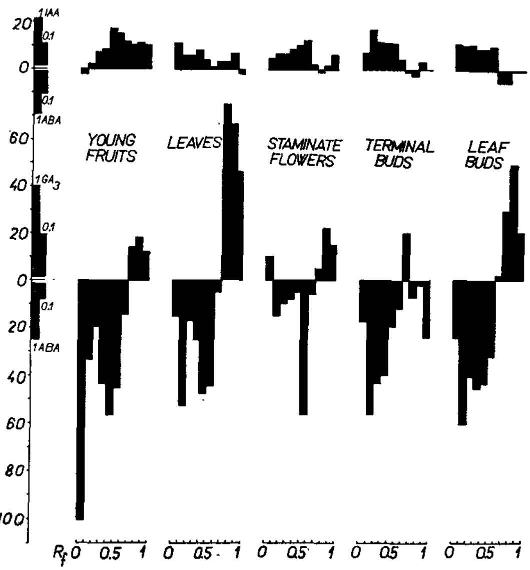


Plate 2. The character of endogenous regulators in young fruits leaves and buds as ascertained at the time of inhibition accumulation (15 July). The upper row represents the values of auxin bioassay, the lower row, those of gibberellin bioassay. The effect of standard samples of IAA, GA_3 and ABA in concentrations 1 and 0.1 p.p.m. is indicated on the ordinate. ▼



c. The Transformation of The Staminate Catkin Into Androgyneous Inflorescence

The spraying of the leaves with higher (0.3%) IAA concentrations damaged the young leaves, as well as the whole stems. The outgrowing of the young staminate catkins and of leaf buds was observed during the following period. The outgrowing catkins in some cases bore pistillate flowers at the basal part and staminate flowers at the apical part. These examples indicate that the young floral primordia are hermaphrodite in nature. The rise in the auxin level favoured the development of pistillate flowers (Fig. 3).

d. The Abnormal Structure of the Pistillate Fruits

We did not as a rule succeed in affecting the differentiation of pistillate flowers by treatment with growth substances. The developmental pattern is evidently stabilized by the high content of endogenous inhibitors during the formation of terminal buds, as well as by the high level of auxins during the differentiation of pistillate flowers. The development of young fruits seems to be more susceptible to damage caused by exogenous treatment. Depending on the developmental stage and MH concentrations, the fruits occurred with changed endocarp, cotyledons and diaphragma. There appeared tripartite endocarp, in some cases, and the number of cotyledonary lobes varied. Seeds with circular cotyledons were observed (Fig. 4).

Discussion

The relation between the growth and differentiation processes and the level of endogenous regulators is evident from the results obtained. These relations are apparently more complicated (Steward 1968) and even the dynamic studies of the character of the growth substances fail to give a clear picture of the regulatory mechanism involved. The data on the differentiation of staminate catkins and pistillate flowers clearly possess general validity and are in conformity with those on sex determination in *Cucumis sativus* (Atsmon and Galun 1962), *Canabis sativa* (Heslop-Harrison 1959), as well as on maize inflorescence development (Sladký 1969).

The histograms illustrate the results of the analyses of whole buds and it is difficult to evaluate the role played by different components of the bud in the pool of growth regulators. Abbott (1970) considers the bud scales in apple as hormonally inactive organs and relate their origin with the decline in cytokinin level and the accumulation of inhibitory activities within the plants.

There is still no clear idea of the nature of the inhibitors as revealed by the two bioassays. The values obtained using standard samples of ABA (1.0 and 0.1 p.p.m.) indicate that both bioassays are sensitive enough to detect the presence of this inhibitor. The assumption ascribing a regulatory role in dormancy to the endogenous inhibitors is based on the work of Thomas *et al.* (1965). These authors bring evidence that dormin isolated from maple and birch is identical with ABA and exerts an antigibberellin activity. These data might help to explain the inhibitions ascertained by the lettuce bioassay (Fig. 2). The occurrence of abnormities in buds with a developmental pattern modified in the preceding year indicate that the application of regulators did not ensure the undisturbed course of development. The different types of abnormities apparently reflect the different modes of regulator effect. The developmental stage of the floral primordia, as well as the concentration of the regulator used are evidently involved in these phenomena. Even this trend was signalled by the observations made on *Campanula* and *Veronica* flowers (Sladký 1966).

The leaf abnormities indicate the fusion of leaflets with the terminal blade, giving rise to a simple leaf. In the sense of DOSTÁL's (1960) recapitulation theory such an oval leaf points to a relationship with the more primitive family of *Fagaceae*. The fact that the abnormities in most cases tend to reflect ancestral forms gives support to the phylogenetical classification of the family *Juglandaceae* prior to *Fagaceae* (TAKHTADZHYAN 1961). This relationship is also indicated by abnormal fruits with circular cotyledons and tripartite endocarp.

The abnormal pistillate catkins show that the flower primordia are hermaphrodite in nature, like for example the flowers of cucumber, maize and hop. Many peculiarities of the morphological structure of walnut fruit may be explained by the inhibitions accompanyig the differentiation of pistillate flowers and fruits. The same explanation holds good regarding the fact that pistillate flowers are the remnant of racemic inflorescence primordia (LEROY 1955). The arrangement of pistillate flowers in long clusters still appears in the form *Juglans californica* WATSON.

The data obtained represent a basis for understanding the differences in the course of walnut bud differentiation and stress the role played in these processes by growth regulators. The potential abilities of young meristems are thus revealed.

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ZDENĚK SLADKÝ, Laboratoř fyziologie a anatomie rostlin přírodovědecké fakulty University J. E. Purkyně, Brno: **Úloha endogenních regulátorů v diferenciačních procesech ořešáku *Juglans regia* L.** — Biol. Plant. 14 : 273—278, 1972.

V práci byl sledován charakter endogenních regulátorů ve vyvíjejících se pupenech, mladých plodech a dospělých listech ořešáku. Pomocí biotestů bylo zjištěno, že v nejmladších základech vegetativních pupenů je poměrně vysoký obsah auxinových a giberelových látek. V dalším vývoji základů v prašníkovou jehnědu poklesá množství látek auxinové povahy. Vývoj listových pupenů je provázen podle ovsového biotestu hromaděním inhibic a podle saláto-

vého testu výraznými stimulacemi. Počáteční vývoj terminálního pupenu je rovněž provázen inhibicemi a teprve následná diferenciace pestíkových kvítků je charakterizována krátkodobým zvýšením hladiny auxinů. V extraktech z tohoto období byly salátovým testem zachyceny inhibice. V polovině léta je v mladých plodech, dospělých listech a odpočívajících pupenech dosti vysoký obsah auxinových látek, ale podle salátového biotestu se hromadí již výrazné inhibice.

Regulační význam růstových látek v diferenciačních procesech je možno lépe posoudit až v druhém roce, kdy pupeny prorůstají a objevuje se řada listových abnormit. Abnormní jehnědy mají různý počet kvítků a tyčinek nebo dokonce nesou i pestíkové kvítky. Vývoj plodů je možno pozměnit v rané fázi diferenciace. Abnormní plody osvětlují řadu strukturálních zvláštností.

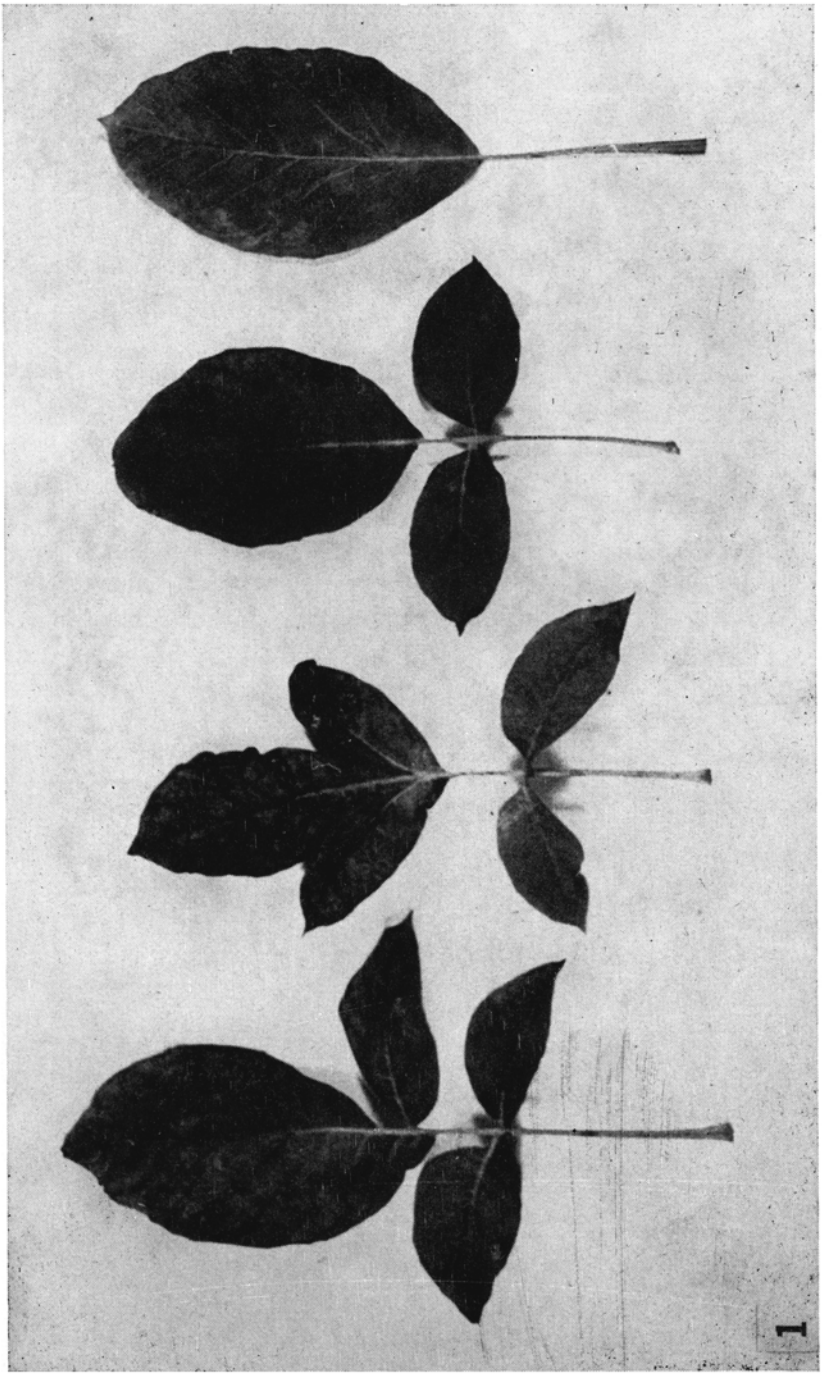

Fig. 1. The abnormal leaves of *Juglans regia*. The fusion of the leaf blade resulting in the origin of a simple leaf is clear.

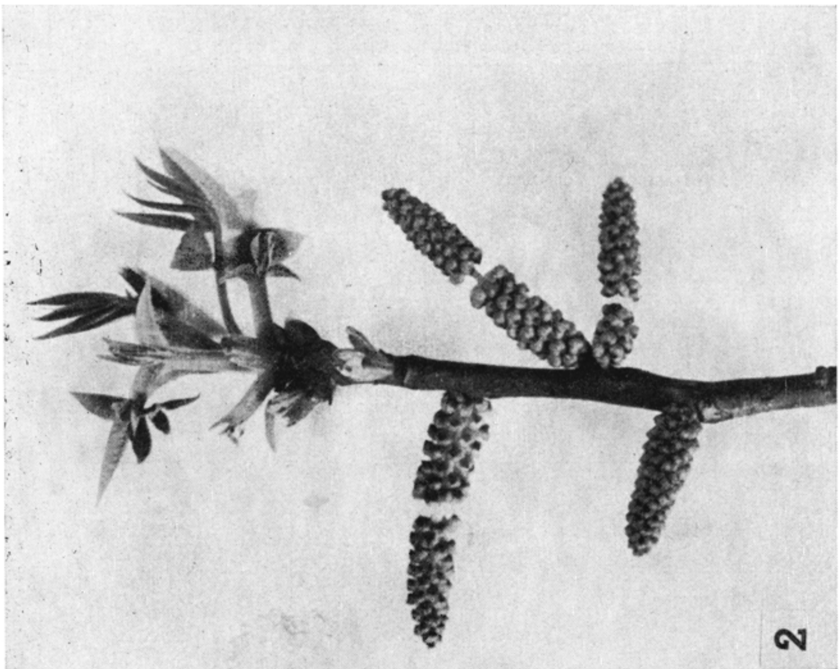

Fig. 2. Treatment with MH brings about the abortion of staminate flowers on the catkin spindle.

Fig. 3. Following the application of IAA, some outgrowing catkins bear pistillate flowers at the basal part.

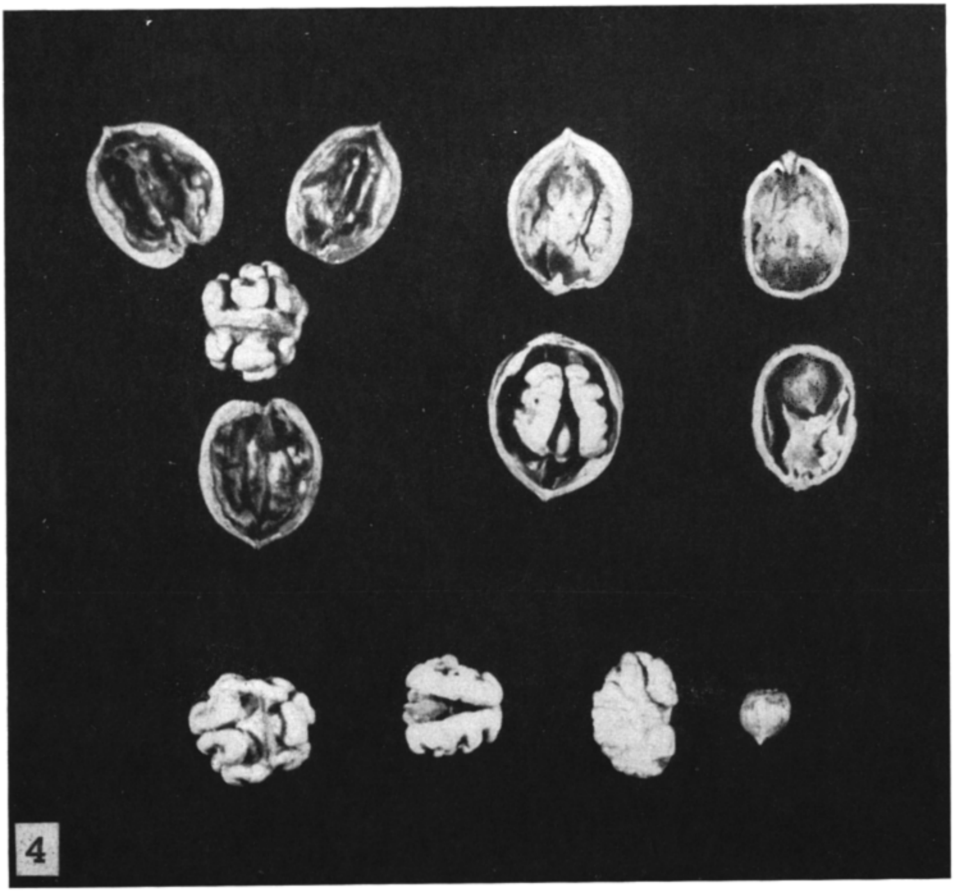

Fig. 4. The application of MH in the course of fruit development results in abnormal seeds.