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Experimental Study of Floral Morphogenesis III. Study of Developmental Possibilities of Leaf and Floral Primordia and the Origin of Fruits in *Juglans regia* L.

Experimentální studie květní morfogeneze III. Studium vývojových možností listových a květních základů a vznik plodů u *Juglans regia* L.

Zdeněk Sladký

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An experimental study of morphological structures revealed a pattern of potential abilities of various paths of young meristem development. The differentiation of staminate catkins takes place in the presence of gibberellin substances which control the sex differentiation of flowers and stimulate their number. Inhibitions in the course of establishment of leaf buds contribute to keeping the vegetative stage. Auxin substances accompany the differentiation of pistillate flowers in the terminal buds and, together with inhibitions, induce the reduction of the number of pistillate flowers, the simplification of the structure, the dormancy of buds, etc. Using suitable synthetic substances it is possible to change the ratio of buds in twigs, control the numbers of flowers in both inflorescences, regulate the drop and the development of fruits. The different ripening of flowers seems to be conditioned by a different quantity of endogenous regulators in the terminal and lateral buds. Spring frosts can impair the development of the ovule and the structure of the embryo sac. Under unfavourable conditions a number of apomictic embryos originate.

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Introduction

Recently there has been a planned extension of walnut planting in this country. In choosing new varieties it is necessary to take into consideration a number of specific properties of floral ecology, resulting from different development of flowers, wind pollination, or the processes of fertilization, origin of embryos and fruit development. Respecting these reproduction peculiarities can favourably influence the height of the future crops.

A great part of these specific processes is in connection with the sensitivity to frost of the walnut. The walnut is native in the warm regions stretching from the Balkan Peninsula and the lower Danube to the Himalayas and Burma in the East. In the course of the last two thousand years of its cultivation it has spread over Greece and Italy to Central and Northern Europe. Polar winters, hitting occasionally also our range, have often destroyed whole stands of walnut trees. The individuals surviving these disasters became parent basis for further generations. The process of surviving and adaptation to rough climatic conditions could take place only thanks to developmental plasticity and rich potential abilities of young meristems. Some potential

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abilities can be experimentally shown even in the present forms. It is evident that these abilities in young meristems are controlled by growth regulators in the course of growth and differentiation (Sladký 1972).

Bud differentiation

On the shooting twigs of the walnut new bud primordia (Plate IX., Fig. 1) arise in the axils of young leaves. In the following period they can develop into buds of three different types. The first buds as a rule develop into primordia of staminate catkins and they continue to develop to mid-May (Plate IX., Fig. 2). In the following period only vegetative buds with primordia of leaves develop (Plate IX., Fig. 3). Towards the close of the growth period terminal buds arise, in which primordia of pistillate flowers are differentiated (Plate IX., Fig. 4). This sequence of the differentiation processes is common in the majority of mature fruit-bearing trees. The development and differentiation of all three types of buds takes only a short time and ceases soon. Further growth and differentiation take part as late as in the following year.

In our previous papers (Langrová et Sladký 1971, Sladký 1972) we tried to clarify the reasons of this varied differentiation of the bud primordia in some old walnuts growing in the Botanical Garden of Brno University. We investigated the level of endogenous growth regulators during the development of different types. At the same time we tried to find out the character of the growth substances in leaves and fruits of different age. The principle of the methods consisted in extracting growth substances from young primordia and chromatographically dividing the extract. The character of endogenous regulators was ascertained by means of specific bioassays. Auxin substances were determined in segments of oats, gibberellins were tested with the hypocotyles of lettuce, and cytokinins were determined from the rate of chlorophyll in segments of barley. By each bioassay we could also find out the presence of inhibitory substances. Thus we obtained a picture of endogenous regulators in various development phases of buds, leaves, inflorescences, and fruits.

The origin and development of the individual bud primordia differs considerably by the content and character of growth regulators. The development of staminate buds is accompanied by outstanding stimulations mainly by substances of gibberellin-like character. The following development of vegetative buds with leaf primordia is characterized by a decrease of substances of stimulative character and by the presence of inhibitions. The differentiation of pistillate flowers in terminal buds is accompanied by an increased content of inhibitions and a short-term increase in auxin substances.

This picture showing a connection between the differentiation processes and growth regulators enables us to understand a number of anatomical and morphological peculiarities (Manning 1938, 1940, 1948), which could not be explained in detail. The following examples will illustrate the case. The stimulative character of endogenous regulators in the differentiation of staminate catkins provides optimum conditions for the realization of potential abilities of young primordia. Gibberellic substances apparently play a determining role in these processes in the differentiation of the staminate sex. The information agrees with literature data in other monoecious plants (Galun 1959).

The presence of inhibitions in leaf buds explains the probable cause of stopping the development processes on the vegetative level. The origin of pistillate flowers in terminal buds is, besides inhibitions, accompanied by a short-term increase of auxin level. The information about the determining role of these endogenous regulators for the differentiation of the pistillate sex agrees with our previous data in *Zea mays* L. (SLADKÝ 1969) and in *Salix caprea* L. (HESLOP-HARRISON 1964). The quantity of accumulated inhibitions apparently controls the number of arising pistillate flowers (Plate X., Fig. 8).

The connection of the differentiation processes with the level of endogenous regulators was verified in further experiments. Using a suitable type of a synthetic regulator we changed the level of endogenous substances, thus regulating the natural course of the development of primordia. The spray of young leaves and twigs with solutions of indole-3-acetic acid increased the origin of leaf buds to the detriment of staminate catkins. By applying triiodobenzoic acid, which acts as antiauxin, a reverse increase of staminate buds took place. Artificially induced inhibitions, e. g., by maleic hydrazide, in the course of the origin of catkin buds caused a reduction of the number of flowers on the catkin spindle and reduced the number of stamens in the flowers (Plate X., Fig. 6). Inhibitions induced in the process of origin leaf buds resulted in a series of leaf abnormalities in the following year (Plate X., Fig. 7).

The fusion of the leaflets into a simple oval leaf, resembling a beech leaf by its shape, is apparent from the photograph. Interpreting these results in the light of the recapitulation theory by DOSTÁL (1960), we could see a simpler feature in the origin of simple leaves.

We did not succeed in overcoming the inhibitions by synthetic stimulators in the course of the differentiation of terminal buds. By means of bioassays we found out that the chief source of natural inhibitions are adult leaves. Gradual defoliation of twigs in some cases resulted in as many as 14 pistillate flowers arising in the terminal bud (Plate X., Fig. 9).

A number of peculiarities of the reduction character in the pistillate flowers, like stunted leaves and leaf scales are the result of accumulated inhibitions at the time of their origin. A raceme of 12 fruits in f. *racemosa* DUH. indicates that a bundle of 2 to 4 pistillate flowers in our types of forms is a remnant of the racemose inflorescence.

Flower ripening

Staminate and pistillate flowers of the walnut do not develop simultaneously. The difference ensures cross-pollination, influences the processes of fertilization and expressively conditions the fruitfulness of the walnut (WOOD 1932). The difference in the time of ripening of staminate catkins and pistillate flowers in the same tree ranges from three to four weeks. This property, though genetically dependent, can be modified by the age of the tree and by climatic conditions. In young walnut trees staminate flowers develop as a rule earlier, whereas in old trees the contrary is the case. To safeguard the processes of fertilization it is advisable to plant together forms yielding sufficient amount of pollen for all ripening stigmas.

To explain the differences in ripening of staminate and pistillate flowers, we also used methods of endogenous regulators. We found out that the proper budding preceded the increase of the level of endogenous stimulations in the meristems, so that already a month before the macroscopic manifestation of the growth it was possible to determine which of the buds would develop sooner. Control by means of exogenous application of growth substances is, however, not at all simple. The growth and shooting of the apical and lateral buds are evidently influenced by a different distribution of plastic substances from the roots.

Apomixis

The processes of fertilization are seriously endangered by spring frosts. It is above all the shooting catkins that are usually damaged by frost; they turn black and sometimes they fall off before they can shed pollen. Even though the pistillate flowers are more resistant, frosts can damage the stigmas which turn black and dry, and make fertilization impossible. Careful observers sometimes wonder why some trees, seriously damaged by frosts during florescence, yield a normal crop. The explanation of this phenomenon was rather difficult, because until recently there was no evidence of apomixis in the walnut (Nygren 1967). Scarce data in the literature did suggest a possibility of the apomictic origin of embryos in the walnut (Zarubin 1949), but an exact verification was missing. Schanderl (1964), using improved methods of isolating pistillate flowers succeeded in verifying the possibility of the origin of seeds without fertilization in various cultivars. Examining the anatomy of pistillate flowers he found out that in some ovules the embryo sac did not develop at all and that the vegetative embryo originated from the inner integument of the nucellus. The fact that apomixis occurs more frequently in walnut trees grown in colder regions and that the percentage of apomictic fruits is different in different years shows that in some cultivars an occasional apomixis may be concerned.

In our work we found out that the number of embryos arisen without fertilization varied between 10 and 20 per cent. It can easily be calculated that this quantity would be sufficient to grant a good crop without fertilization. An anatomical study demonstrated that a great majority of ovules contained a normal embryo sac in the nucellus (Plate X., Fig. 9, Plate XI., Fig. 10). Spring frosts can, however, damage the normal course of the development of the ovule and the organization of the embryo sac. In some cases we also found a nucellus without any embryo sac or with only a group of large cells (Plate XI., Figs. 11 and 12). Another time two vestigial embryo sacs could be observed.

The analyses of the content of growth substances in experimentally cooled leaves (−4° C) showed that the lowered temperature brought about certain changes in the level of endogenous regulators. They can be one of the causes of the origin of vegetative embryos.

We verified the role of growth substances in the processes of origin of embryos without fertilization in the endogenous application. A young stigma was smeared with lanoline paste with 1 per cent of IAA, GA_3 and KIN. Kinetin and IAA seem to have supported the origin of fruits. It is, however, difficult to discuss their direct influence in the origin of the embryo.

Apomixis is extremely important in evolution and plant breeding. Planters used to take seeds from trees which, in spite of occasional spring frosts, yielded a regular crop. This was, unintentionally, a selection of the apomictic origin of embryos. New seedlings as a rule keep the properties of the mother tree.

Fruit development

The development of young fruits passes through a critical period in the latter half of June, when a mass fruit drop occurs. Analyzing both the growing and the dropped fruits we found that the latter contained fewer auxin substances and inhibitions were gathering in them. These conditions reflect the same situation in the leaves. This is in agreement with literature data on the causes of fruit drop in apple trees (Luckwill 1953). The possibility of decreasing the percentage of fruit drop was verified by spraying the leaves with IAA solutions in the concentration of 100 mg per 1 litre.

One can make sure about the possibilities of different paths of development of young fruits by a suitable application of inhibiting substances, e.g., by the solutions of maleic hydrazide. The strength of intervention and the stage of fruit development result in abnormalities of different qualities (Plate XI., Fig. 14). In some cases the shell consisted of three parts, the cotyledons had only three or even two lobes. A few embryos with circular cotyledons, resembling those of the hazel, could also be observed. If these abnormalities point to ancestral forms, they could support the idea of *Juglans* being phylogenetically younger than the members of the family *Corylaceae*.

We can make sure about the inhibition function of the leaves even before the close of vegetation by removing the leaves from twigs in August. Terminal buds start growing and new leaves and pistillate flowers develop. Fig. 15 (Plate XI.) shows such a case in which, besides the ripening fruits, we obtained pistillate flowers for further experiments.

Souhrn

Experimentální studium morfologických struktur odhaluje škálu potenciálních schopností různého směru vývoje mladých meristémů u *Juglans regia* L. Diferenciace prašníkových jehněd probíhá za přítomnosti giberelových látek, které usměrňují pohlaví květů a stimulují jejich počet. Inhibice v průběhu zakládání listových pupenů přispívají k udržení vegetativního stavu. Auxinové látky provázejí diferenciaci pestíkových květů v terminálních pupenech a spolu s inhibicemi navozují redukci počtu pestíkových květů, zjednodušení stavby, dormanci pupenů apod. Vhodnou aplikací syntetických látek je možno měnit poměr pupenů na letorostech, usměrňovat počet květů v obou květenstvích, regulovat opad a vývoj plodů. Zdá se, že rozdílné dozrávání květů je podmíněno rozdílným množstvím endogenních regulátorů v terminálních a laterálních pupenech. Jarní mrazíky mohou narušit vývoj vajíčka a organizaci zárodečného vaku. Za nepříznivých podmínek dochází ke vzniku určitého množství apomiktických embryí.

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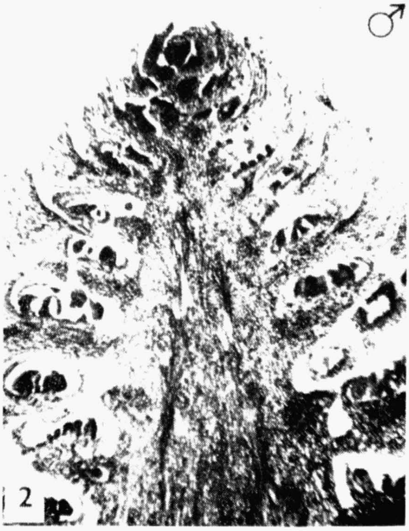
See also plates IX.—XI. in the Appendix.



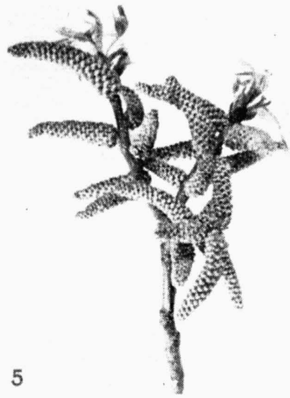
MAY / GIBBERELLINS

JUNE / INHIBITIONS

JULY / AUXINS

Plate IX. — Study of potential abilities of growth and development in bud primordia of *Juglans regia* L. — Fig. 1. — Growing apex of a young bud is the basis for the origin of all three types of buds. — Fig. 2. — In May staminate buds arise under the presence of gibberellin substances. — Fig. 3. — In June inhibitions accompany the origin of leaf buds. — Fig. 4. — In July pistillate flowers are differentiated in terminal buds under the influence of auxin-like substances.

Z. Sladký: Experimental Study of Floral Morphogenesis III. Study of Developmental Possibilities of Leaf and Floral Primordia and the Origin of Fruits in *Juglans regia* L.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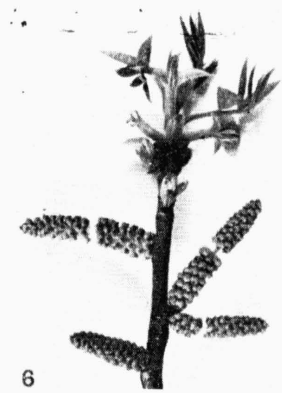
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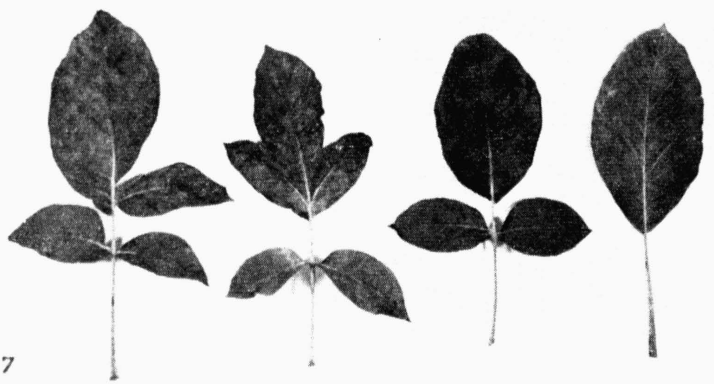
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Plate X. — Control of developmental processes in the following year. — Fig. 5. — A quicker development of staminate flowers is conditioned by the increase of stimulating substances in catkins. — Fig. 6. — Inhibitions induced in the course of catkin primordia differentiation result in the reduction of the number of flowers and stamens. — Fig. 7. — Inhibition intervention into the development of leaf buds results in the fusion of leaflets into a simple leaf. — Fig. 8. — A quicker development of pistillate flowers is induced by increasing the level of stimulating substances in terminal buds. — Fig. 9. — A reduction of the content of inhibitions in the period of pistillate flowers by means of defoliation results in increasing their number.

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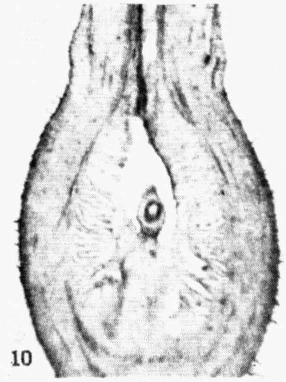

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Plate XI. — Origin and development of walnut fruit. — Fig. 10. — Most pistillate flowers contain one direct ovule with embryo in nucellus. — Fig. 11. — Embryo sac is of oval shape with egg cell, synergid and antipodal cells. × 120. — Fig. 12. — Spring frosts impair the development of staminate flowers and a nucellus devoid of embryo sac develops. — Fig. 13. — Another time there are only several large cells in the place of the embryo sac. × 120. — Fig. 14. — The development of fruits can also be altered so that fruits with tripartite shells originate, the number of cotyledonary lobes changes and a seed with circular cotyledons arises. — Fig. 15. — Following the removal of inhibitions from the leaves, terminal buds with pistillate flowers grow side by side with ripe fruits.

Z. Sladký: Experimental Study of Floral Morphogenesis III. Study of Developmental Possibilities of Leaf and Floral Primordia and the Origin of Fruits in *Juglans regia* L.