ACTA SOCIETATIS
BOTANICORUM POLONIAE
Vol. XLVII, nr 1—2
1978

# Hormonal balance in apical meristems of Scots pine shoots as a very early symptom of flower sex differentiation*

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(Received: November 30, 1977)

Abstract

The investigations were conducted on the embryonic shoots which were taken at the stage just before the start of the elongation of the last year formed embryonic shoot; it means long time before male and female cone primordia initiation. The results point out to the existence of distinctive balance of auxins to gibberellins in apical meristems of potentially female and male shoots for about a month before the initiation of new embryonic shoots. These results confirm our previous suggestions that femaleness and maleness in Scots pine is correlated with a high amount of auxins and gibberellins, respectively.

## INTRODUCTION

The previous investigations (Kopcewicz et al., 1977) showed that pine shoot apical meristems, growing on the position of tree crown that favours male flower production, are characterized by the higher level of gibberellins than the shoot apical meristems formed on the position that favours female flower differentiation. On the other hand the female shoot meristems contained much more auxins than the male ones. The differences between auxin and gibberellin levels were observed at the stage of elongation of the last year embryonic shoots.

The purpose of present investigations was to establish whether the differences between the hormonal levels of both the potentially female and male embryonic shoots exist already at the earlier stages of their development.

* Research supported in part by grant from the US Department of Agriculture, Agricultural Research Service (FG-Po-296).

## MATERIAL and METHODS

The analysis of the growth hormones were undertaken on the material from the same group of about sixty year old Scots pine (*Pinus silvestris* L.) trees as it was in the previous investigations (Kopcewicz et al., 1977). Embryonic shoots at the stage just before its elongation were taken as a sample for investigation (April 28, 1976 — Fig. 1,I). The male archespor cells were at the stage of early meiotic prophase (till zygotene). In the female cone cells only mitoses were observed. Mitoses were observed also in dwarf shoot primordia and sometimes in the peripheral meristems zones of the apical meristems.

The embryonic shoots were taken from both: the branches producing about 90-95% of male cones and from the branches producing about 65 - 80% of female ones. Then these embryonic shoots were divided into three main groups (Fig. 2):

A. embryonic shoots with female cones
B. embryonic shoots without female cones but formed on the position that favoured female flower production
C. embryonic shoots with male cones

Consistently embryonic shoots without male cones but formed on the position that favoured male flower production should also be used. It was impossible, however, because of their scarce occurrance.

The embryonic shoots were dissected into two sections (Fig. 2) and growth hormones were extracted separately from apical meristems and basal parts.

For the determination of the development stages 10 embryonic shoots of each group (A, B and C — Fig. 2) were taken as a sample. These samples were fixed and stained as before (Kopcewicz et al., 1977).

Plant growth hormones (auxins, gibberellins, cytokinins and abscisic acid-like inhibitors) were isolated from 500 embryonic shoots according to the methods previously described (Kopcewicz et al., 1977).

## RESULTS and DISCUSSION

There are only scarce informations concerning the participation of growth hormones in the flower sex differentiation (Hashizume, 1966; Pharis and Morf, 1970; Pharis et al., 1975; Pharis, 1975). The results of our previous experiments (Kopcewicz et al., 1977) suggest that an endogenous equilibrium of auxins to gibberellins is involved in the mechanism of Scots pine flower sex differentiation similarly as in the case of herbaceous plants (Atsmon et al., 1968).

The above mentioned experiments were carried out at the stages just before the initiation of the axillary buds differentiating later into male or female inflorescences (Fig. 1, II). The present investigations were conducted on the embryonic shoots which were taken at the stage just before the start of the last year formed embryonic shoots elongation (Fig. 1,I) — it means long time before male and female cone primordia initiation.

The results of the experiments on auxins show that only the female embryonic shoots with inflorescences contain high amounts of

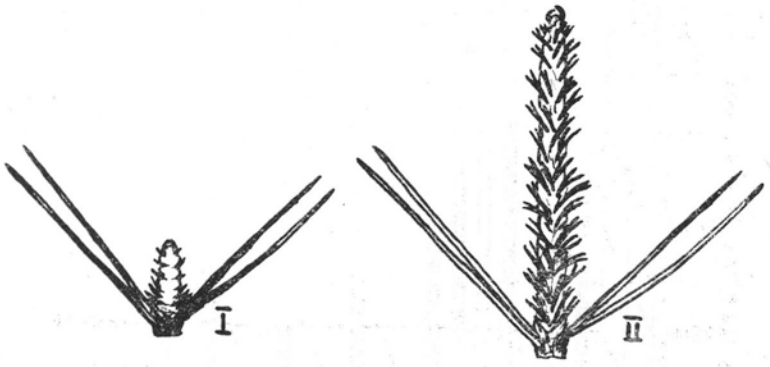


Fig. 1 Stages of the investigated Scots pine embryonic shoots. I. The last year embryonic shoot at the stage just before elongation (about one month before the new embryonic shoot initiation). The embryonic shoots at this stage of development were used in the present investigations. II. The pine embryonic shoot at the stage of elongation (just before the new embryonic shoot initiation). Apical meristems from these embryonic shoots were used as the earliest studied phase in the previous investigations (K o p c e w i c z et al., 1977)

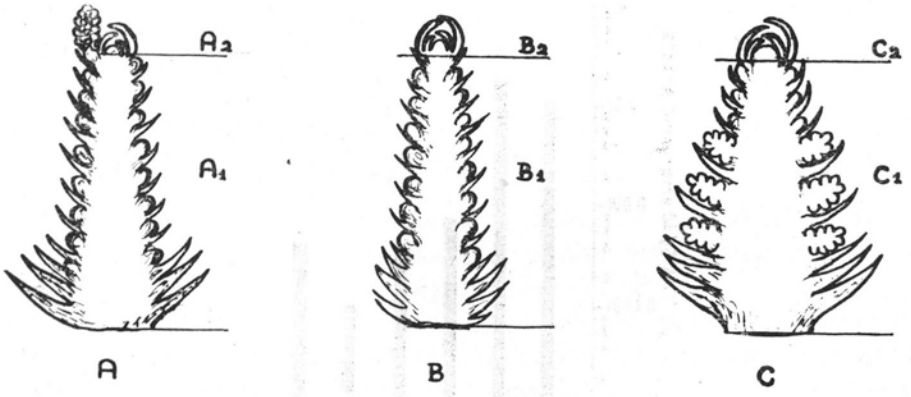


Fig. 2 Three groups of embryonic shoots used in the experiments

A — A female embryonic shoot with the cone primordium; B — An embryonic shoot growing on the position of the pine tree crown that favours the production of female inflorescences; C — A male embryonic shoot with male cone primordia.
$A_1$, $B_1$, $C_1$ — basal part, $A_2$, $B_2$, $C_2$ — apical meristem

those compounds (Fig. 3, $A_1$ and $A_2$). Especially big amounts of auxins were observed in the apical meristems ($A_2$) even in comparison with the basal parts of these embryonic shoots ($A_1$). It is essential because of the fact that as the criterion of quantitative evaluation of hormonal substances a number of analysed apical meristems and basal parts of embryonic shoots were taken. The weight of the basal parts was many

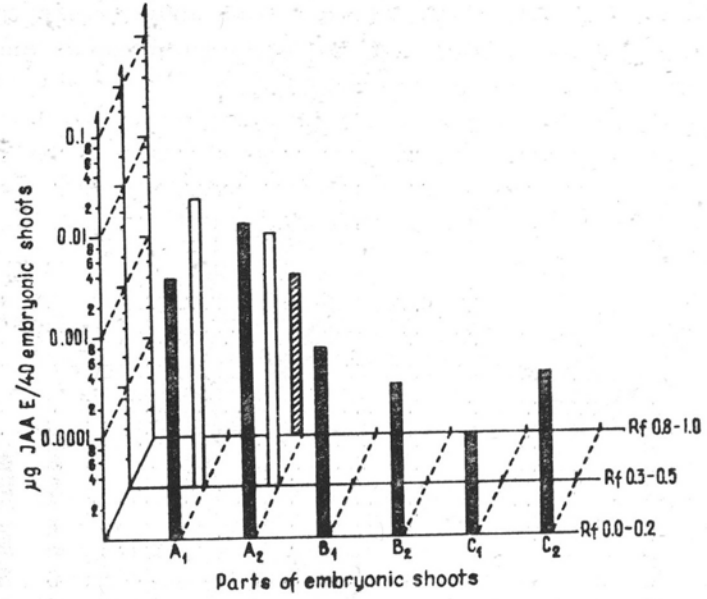


Fig. 3 Auxin-like substances in apical and basal parts of female and male embryonic shoots

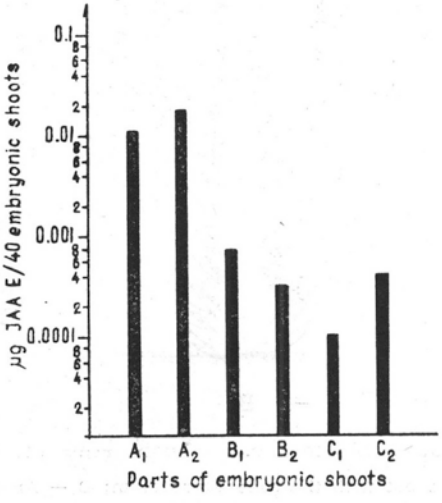


Fig. 4 Total amounts of auxin-like substances in apical and basal parts of female and male embryonic shoots

times (15—20) greater than the weight of apical meristems. So in calculation per weight the observed differences would be even greater. It seems to be interesting that the increased amounts of auxins occur merely in embryonic shoots with female cones, whereas those without inflorescences contain small amounts of auxins — similarly as male embryonic shoots (Fig. 4). On the chromatograms from apical meristems of female embryonic shoots ($A_2$) three groups of auxin-like substances were found. Basal parts ($A_1$) contain two groups of these compounds (Fig. 3). Both the localization and the earlier studies on the identification of auxins in Scots pine shoots (W o d z i c k i, 1968) allow to assume that at Rf 0.3—0.5 zone IAA is the main active compound. It seems, however, that the more detailed identification studies, especially about two additional auxin-like substances which occured in female embryonic shoots, should be desired in future.

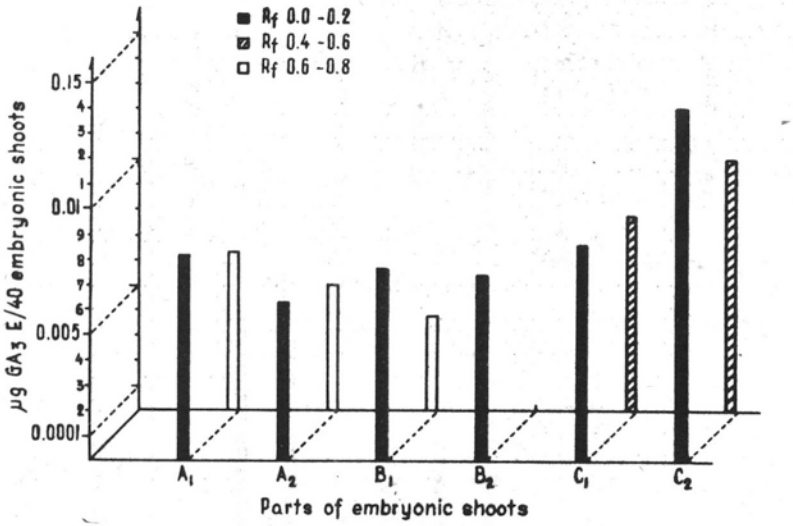


Fig. 5 Gibberellin-like substances in apical and basal parts of female and male embryonic shoots

The results of the experiments on gibberellins show that the highest level of these compounds is observed in the male embryonic shoots, especially in the apical meristem parts (Fig. 5 and 6 —$C_2$). At this stage of development all the embryonic shoots are just before the elongation so the observed levels of gibberellins in the female embryonic shoots both with or without inflorescences may be explained as the "preparation" to enhancing growth processes. Taking into account the above mentioned fact that the apical meristem parts are much smaller than the basal parts of embryonic shoots, the gibberellin level in the apical meristems of all the groups of embryonic shoots is quite high. On the

chromatograms from both the female and male embryonic shoots two groups of gibberellins have been stated (Fig. 5). More detailed physico--chemical and physiological properties of the gibberellins occuring in pine shoots were presented previously (K o p c e w i c z, 1968).

The results of investigations on cytokinins show that the meristems of all groups of the embryonic shoots contained too small quantities of these substances to express some noticeable differences among them (Fig. 7). It is probably connected with the fact that at the investigated stage of development the apical meristems are nearly non-active. The basal parts of the female embryonic shoots are characterized by the higher level of cytokinins than the male ones. It seems that it is related with the mitotic processes observed most often in the cells of female cones and in dwarf shoot primordia. These primordia are more numerous on the female than on the male embryonic shoots. The obtained results seem to support the earlier suggestions (K o p c e w i c z et al., 1977) according to whih the levels of cytokinins in pine embryonic shoots are correlated to mitoses and that cytokinins are most probably not involved in the pine flower sex differentiation.

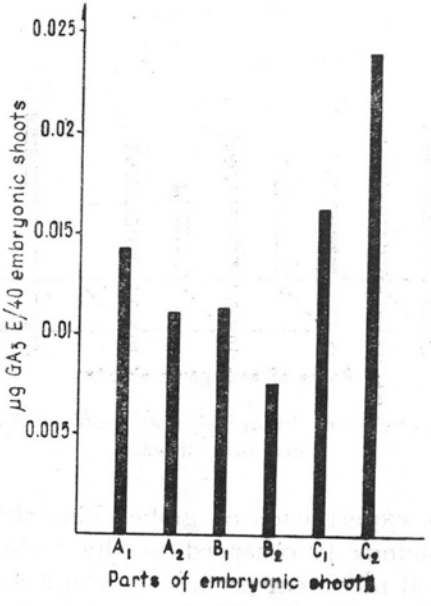


Fig. 6 Total amounts of gibberellins in apical and basal parts of female and male embryonic shoots

The results of the investigations on abscisic acid-like inhibitors show that these substances are contained both in apical meristems and basal parts of all the groups of the investigated embryonic shoots (Fig. 8). Because apical meristems are much smaller than the basal parts,

however, it is possible to expect that the abscisic acid-like inhibitors are in bigger quantities in the apical meristems than in the basal parts. It seems that it is correlated with the fact that investigated apical meristems were almost non-active, whereas basal parts of embryonic shoots are just before the elongation.

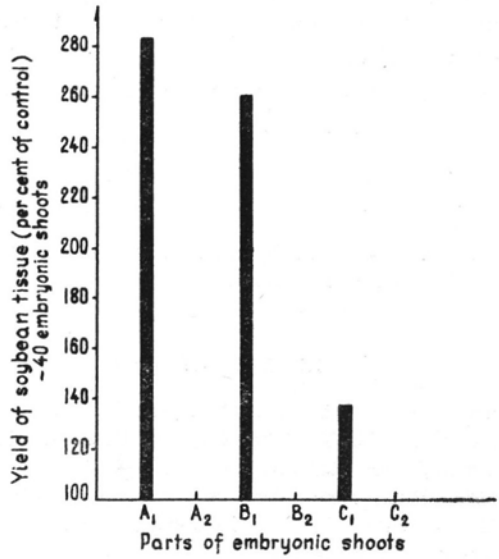


Fig. 7 Cytokinin-like substances in apical and basal parts of female and male embryonic shoots

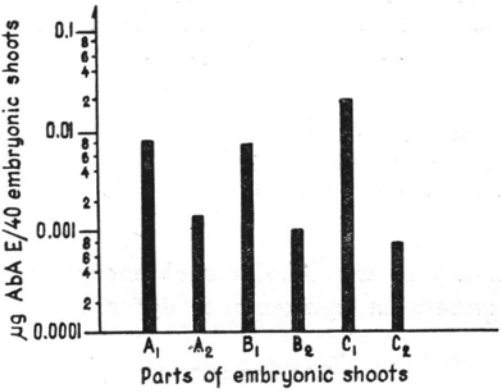


Fig. 8 Abscisin-like inhibitors in apical and basal parts of female and male embryonic shoots

On the basis of above described results it seems that the more pronounced differences between male and female embryonic shoot apical meristems are in the levels of auxins and gibberellins. It is especially interesting to note that these differences exist for such a long time

before the new embryonic shoot initiation. It is known that endogenous auxins levels are very high in female cones of *Cryptomeria japonica* and some species of *Pinus* (Griertych and Forward, 1966). In regard to this it seems possible that the presence of actively growing female cones on some embryonic shoots could promote accumulation of auxins both in the apical and basal parts of these female embryonic shoots. The obtained results also seem to support the earlier suggestions (Kopcewicz et al., 1977) that there is lack of direct correlation between the content of cytokinins or abscisic acid-like inhibitors and flower sex differentiation in Scots pine.

Acknowledgments

We wish to thank Professor M. Michniewicz for stimulating discussions and valuable suggestions during the course of this work.

REFERENCES

Atsmon D., Lang A., Light N., 1968. Plant Physiol. 43: 806—808.
Giertych M., Forward D. F., 1966. Can. J. Bot. 44. 717—738.
Hashizume H., 1966. Chem. Regul. Plants 1: 148—156.
Kopcewicz J., 1968. Bull. Acad. Pol. Sci. ser. biol., 16: 667—672.
Kopcewicz J., Zatorska Z., Kulikowska H., Szcześniak T., 1977. Acta Soc. Bot. Polon. 46: 119—128.
Pharis R. P., 1975. The Forestry Chronicle 51: 1—5.
Pharis R. P., Morf W., 1970. Zesz. Nauk. Uniw. M. Kopernika, Toruń 13: 85—89.
Pharis R. P., Wample R. L., Kamieńska A., 1975. Management of Lodgepole Pine Ecosystems Extension Serv., Pullman WA 99163: 106—134.
Wodzicki T. J., 1968. Amer. J. Bot. 55: 564—571.

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## *Balans hormonalny w wierzchołkowych merystemach pędów sosny zwyczajnej wczesnym symptomem dyferencjacji płci kwiatów*

Streszczenie

Badania prowadzono na zawiązkach pędów w okresie poprzedzającym elongację ubiegłorocznych pędów embrionalnych a więc na długo przed inicjacją męskich i żeńskich kwiatostanów. Wyniki wskazują na istnienie specyficznego balansu auksyn do giberelin w merystemach wierzchołkowych potencjalnie męskich i żeńskich pędów na około miesiąc przed inicjacją nowych pędów embrionalnych. Wyniki potwierdzają nasze wcześniejsze sugestie, że u sosny zwyczajnej żeńskość związana jest z podwyższoną zawartością auksyn w tkankach, zaś męskość z podwyższoną ilością giberelin.