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Gibberellin-like substances in embryonic shoots of Scots pine in relation to generative differentiation

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Abstract

Investigations were carried out on embryonic shoots of lateral and terminal buds of Scots pine (*Pinus silvestris* L.) of different age, at various stages of seasonal development. The results show that there is a correlation between gibberellins content and apical dominance in pine. More are found in terminal buds than in lateral ones. The transition from a juvenile phase into a mature one in Scots pine is accompanied by a lowering of the gibberellin content. The formation of male inflorescences is accompanied by an increased amount of endogenous gibberellins and the appearence of a new group of non-polar gibberellin-like substances. The formation of female cones is connected with the lowering of endogenous gibberellin content.

Key words: pine, embryonic shoots, gibberellins, generative differentiation

INTRODUCTION

Scots pine belongs to trees with heteroblastic development, where the morphology of a juvenile phase differs distinctly from a mature one. Young, vegetative trees have a conical shape, characteristic for a strong-apical dominance, covering the whole tree. Flowering trees, over 20 years old, already have a different shape. The tree crown is made up of branchy shoots and it is not distinctly conical. So, it is interesting to know, whether there is a relation between the elimination of apical dominance and the transition to the generative phase and what is the role of natural growth substances in these processes.

The results of investigations conducted in previous years on the role of plant hormones in the flower sex differentiation of Scots pine (Kopcewicz

et al. 1977, 1978. Kulikowska et al. 1978) suggested the participation of endogenous gibberellins in the control of differentiation of embryonic shoots in the direction of maleness in trees over 60 years old. However, there were no detailed investigations on the role of these substances in the formation of male and female flowers in Scots pine trees of different age groups, and also on the role of gibberellins in the transition of pine from the vegetative phase to the generative one. Thus, the aim of the present work was to study the changes in the content of endogenous gibberellins in the buds of Scots pine differentiating generatively.

MATERIAL AND METHODS

The material for the investigations was the embryonic shoots taken from lateral and terminal buds of pine trees at different ages. In case of flowering trees, the material was taken from male and female parts of a tree crown. In female tree crown regions the branches formed 65-80% of female cones, whereas in the male tree, crown regions the branches formed 90-95% of male inflorescences.

Embryonic shoots were taken from the following groups of trees:

I. 5 year old trees, not flowering;
II. 20 year old trees, not flowering;
III. 20 year old trees, forming only female cones;
IV. 20 year old trees, forming only male flowers;
V. 40 year old trees, flowering;
VI. 60 year old trees, flowering.

All of the trees grew under similar biotopic conditions, on natural stands, on the grounds of the Olek Forestry, Barbarka Wood near Toruń.

Embryonic shoots were taken at the following stages of seasonal development:

I. the stage just before the elongation of embryonic shoots formed during the previous year (the beginning of May);
II. the stage before the primordia initiation of male inflorescences (the end of May or the first half of June, depending on atmospheric conditions);
III. the stage just before the primordia initiation of female cones (the end of June);
IV. the stage during which the processes of enlarging of embryonic shoots take place (the beginning of July);
V. the stage during which the inhibition of cell division processes gradually takes place in embryonic shoots as well as the transition of buds into the state of dormancy (the end of September).

Embryonic shoots were taken from lateral and terminal buds. The plant

material (500 embryonic shoots in every variant of experiment) was extracted using 80% methanol and fractionated according to the method described previously (Kopcewicz et al. 1977). Gibberellins were partitioned by thin-layer chromatography using chloroform: acetic acid: ethyl acetate (90:5:10 v/v) as the solvent system. The lettuce hypocotyl test was used for the determination of gibberellin activity (Frankland and Wareing 1960). The content of gibberellins was established from a standard curve, drawn on the basis of the influence of several concentrations of gibberellic acid on the growth of lettuce hypocotyls. The amount of hormones characteristic for a given developmental stage was calculated as a mean value from the conducted experimental repetitions, and was given as an equivalent of gibberellic acid for 50 embryonic shoots. It was assumed that each bar on the diagram denoted the total content of gibberellins from the neighbouring chromatogram segments, calculated for the proper amount of original plant material. The results of the experiments were analysed statistically and the LSD (Least Significant Difference) was calculated with the significance level $\alpha = 0.05$ and $\alpha = 0.01$. The vertical bars below LSD values are the graphic illustration of the LSD.

RESULTS AND DISCUSSION

The obtained results are a part of investigations on the role of plant hormones in the transition of Scots pine from a juvenile phase to a mature one, with regard to apical dominance and the role of these substances during the differentiation of male and female pine flowers.

The first stage of these investigations was carried out on the material collected during the phase of enlarging the embryonic shoots (the beginning of July). Three groups of trees were taken for the investigations:

1. 5 year old trees, not flowering,
2. 20 year old trees, not flowering,
3. 40 year old trees, flowering.

In the first two groups, a distinct apical dominance in the tree shape was clearly marked. In the third group embryonic shoots were taken from male and female regions of a tree crown.

The results of investigations on gibberellins (Fig. 1) show that these substances are localized on the chromatograms in three active zones: R_f 0.0-0.2, R_f 0.4-0.6 and R_f 0.8-1.0 (in the case of 5 year old trees and male regions of the tree crown of 40 year old trees).

Young, 5 and 20 year old trees (Fig. 2) contain considerably more gibberellins in terminal buds than in lateral ones. It must be stressed

here, that in the case of 20 year old trees this difference is statistically significant. The highest level of gibberellins in terminal and lateral buds occurs in the case of 5 year old trees. A distinctly higher amount of gibberellins in the terminal buds of young trees in comparison with 40 year old trees would suggest the participation of these hormones in the regulation of apical dominance, which may mean acting with auxin in the inhibition

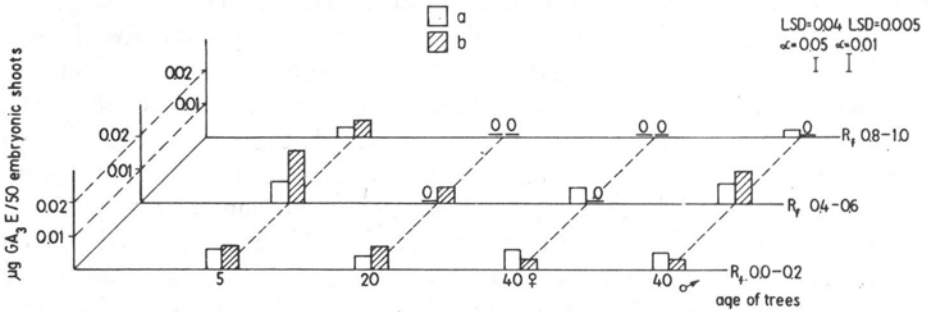

Fig. 1. Gibberellin-like substances in lateral (a) and terminal (b) embryonic shoots of Scots pine trees at different age

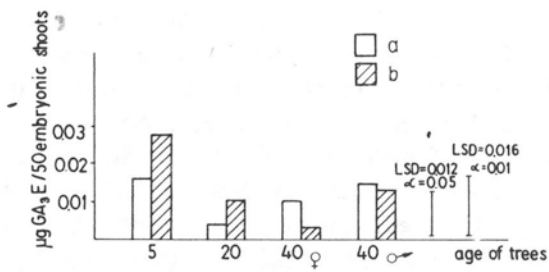

Fig. 2. Total amount of gibberellin-like substances in lateral (a) and terminal (b) embryonic shoots of Scots pine

of the development of lateral buds. Similar results were obtained by Tomaszewski (1970). So, the transition from a juvenile phase into a mature one would be connected with the lowering of the gibberellin level. Galoch and Michniewicz (1978) stated that the transition into the generative stage in Scots pine was connected with an increase in the gibberellin content. Their experiments however, concerned only young, about 15 year old Scots pines. Kopcewicz et al. (1967) stated that flowering 40 year old Scots pine trees contained more gibberellins than young (2 and 10 year old) and old (90 and 140 year old) trees. These discrepancies may be due

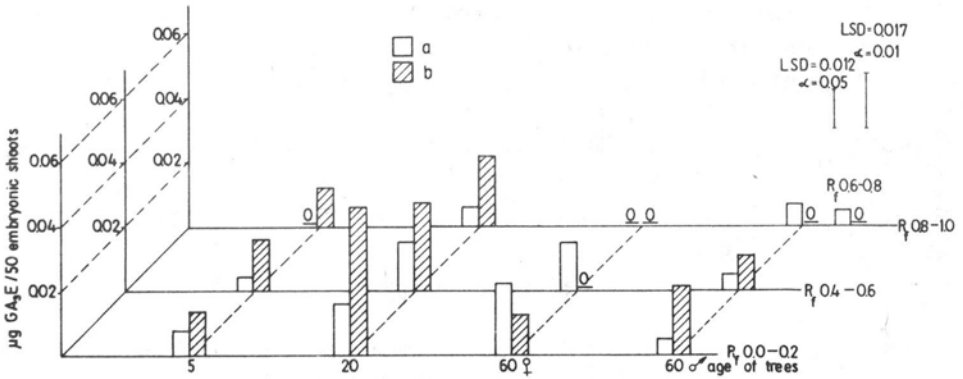

Fig. 3. Changes in the amount of gibberellin-like substances in lateral (a) and terminal (b) embryonic shoots of Scots pine in early May

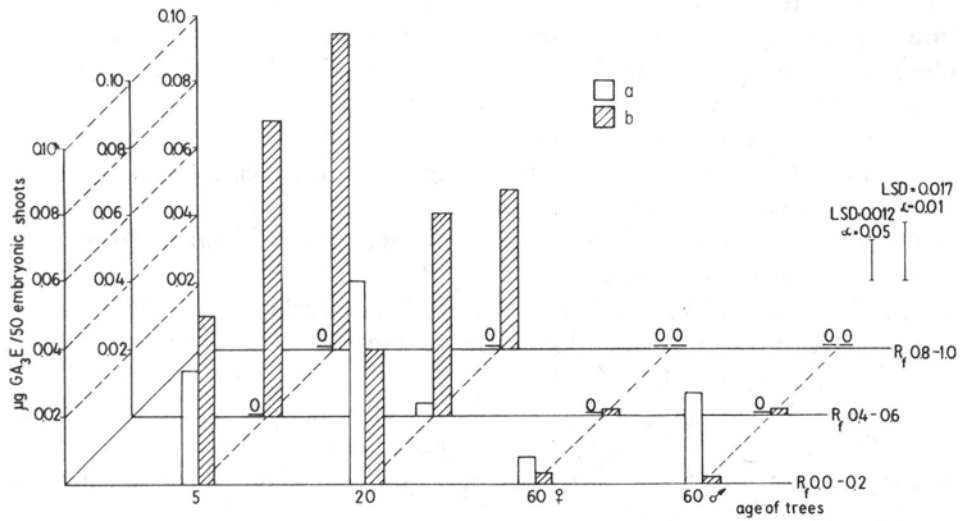

Fig. 4. Changes in the amount of gibberellin-like substances in lateral (a) and terminal (b) embryonic shoots of Scots pine at the end of May

to the different material used (trees of different age and only vegetative buds and shoots were used) and to the fact that the experiments mentioned above did not emphasize transition from the vegetative phase to a generative one, but concerned the dynamics of gibberellins in the ontogeny of Scots pine. On the other hand, Dunberg (1974) maintains that there are no qualitative or quantitative differences in the occurrence of gibberellin-like

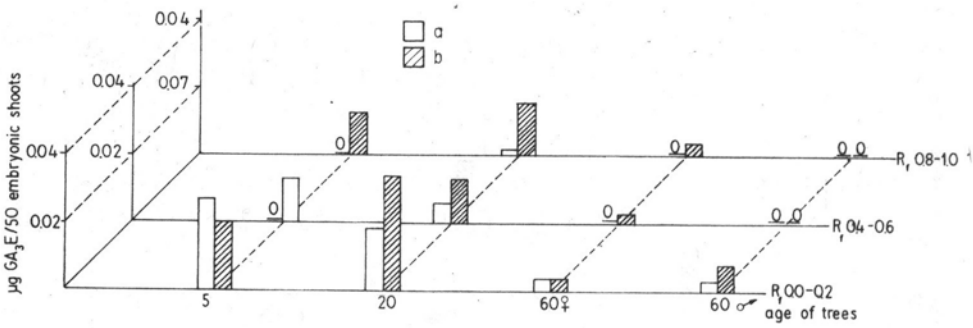

Fig. 5. Changes in the amount of gibberellin-like substances in lateral (a) and terminal (b) embryonic shoots of Scots pine in July

substances between juvenile and mature trees of the Norway spruce (*Picea abies* (L.) Karst.).

In order to make a detailed study of the role of endogenous gibberellins in the transition from the juvenile phase into the mature one in the Scots pine, further investigations were carried out on the material from three age groups of trees at various stages of seasonal development. The object of investigations were embryonic shoots from lateral and terminal buds of the following groups of trees:

1. 5 year old trees, not flowering;
2. 20 year old trees, still not flowering, but just before the transition to the generative phase;
3. 60 year old trees, flowering, where the material was taken from male and female tree crown regions.

The embryonic shoots were taken during three developmental stages:

1. the stage just before the elongation of the last year embryonic shoots (the beginning of May),
2. the stage before the primordia initiation of male inflorescences (the end of May),
3. the stage in which the processes of embryonic shoot enlargement take place (the beginning of July).

The experimental results showed that gibberellin-like substances were localized on the chromatograms in three active zones: R_f 0.0-0.2, R_f 0.4-0.6 and R_f 0.8-1.0 (Figs. 3, 4, 5). Only in the first developmental stage in male embryonic shoots was the fourth active zone R_f 0.6-0.8 (Fig. 3) observed. Five year old trees had more gibberellin-like substances in terminal buds than in lateral ones (Fig. 6) and these differences were statistically significant. At stage 2, a rapid increase in the gibberellin content was observed, mainly in terminal buds. At stage 3, the level of these hormones decreased and was similar to their content in phase 1. Twenty year old trees also

contained more gibberellins in embryonic shoots of terminal buds (Fig. 6) and their greatest amount was observed at stage 2. Generally speaking, young trees, 5 and 20 year old, displayed a higher level of these substances

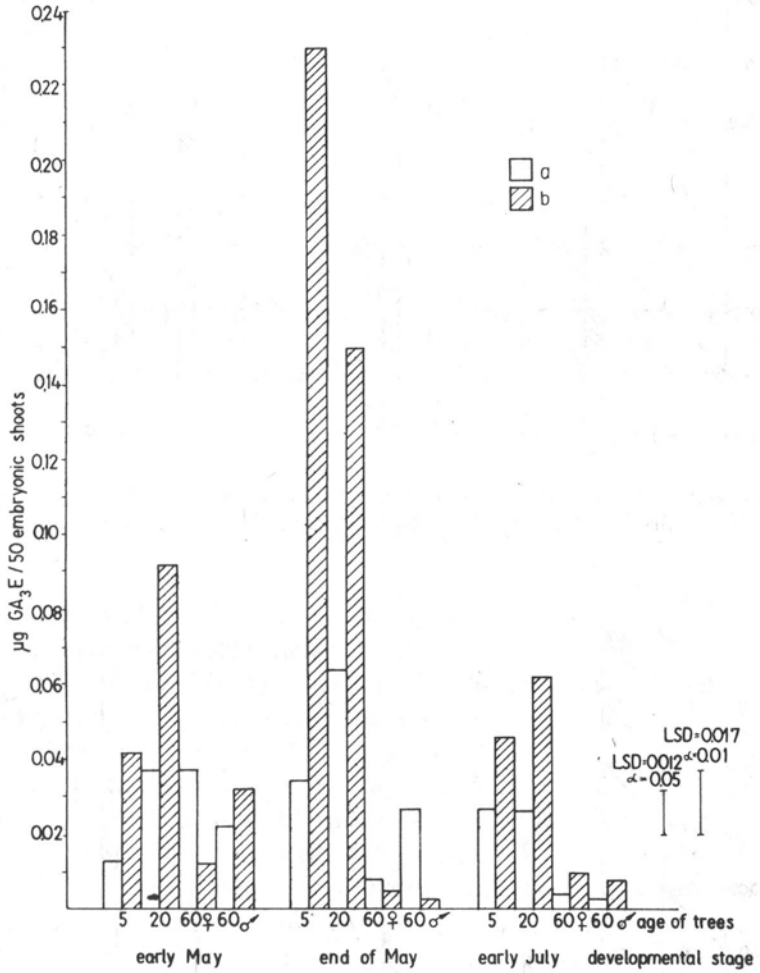

Fig. 6. Total amount of gibberellin-like substances in lateral (a) and terminal (b) embryonic shoots of Scots pine at various stages of seasonal development

than older trees, and more of them were found in terminal buds than in lateral ones. This may suggest the participation of gibberellins in the regulation of apical dominance. Also, it seems that the transition into the generative phase of Scots pine is accompanied by a general lowering of the gibberellin level in embryonic shoots.

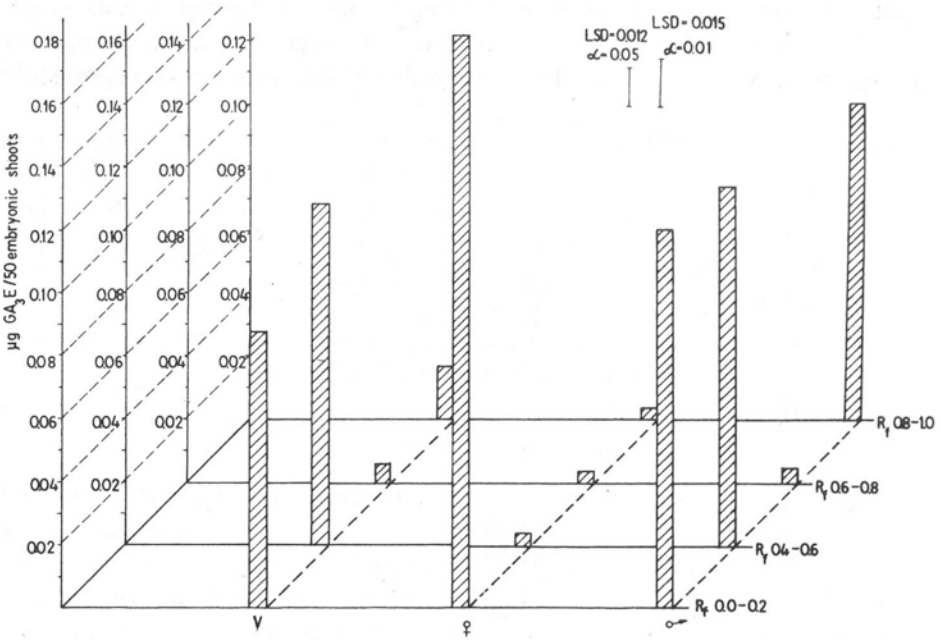

Fig. 7. Gibberellin-like substances in terminal embryonic shoots of 20 year old Scots pine trees in the first half of June. V — vegetative trees, ♀ — female trees, ♂ — male trees

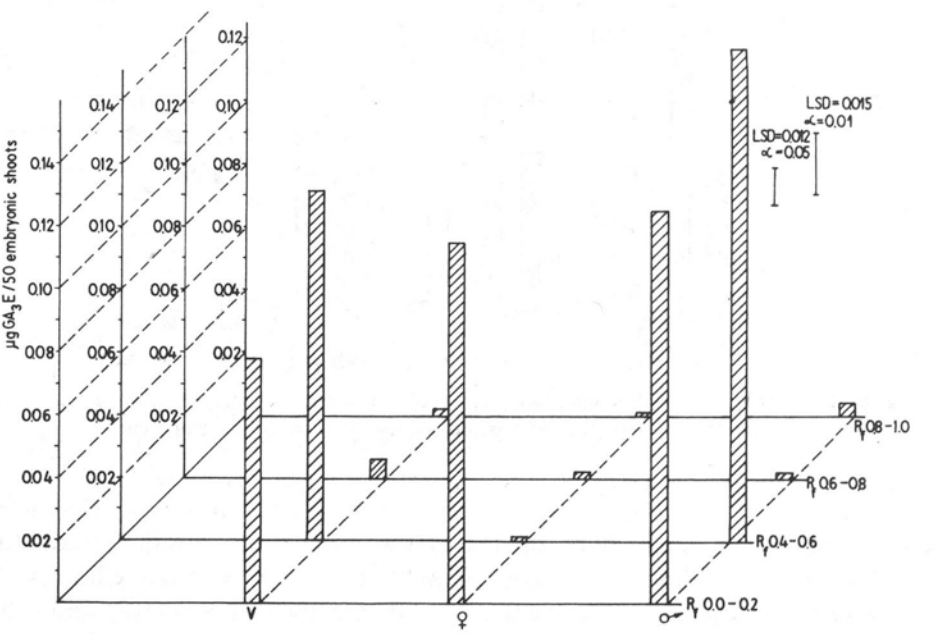

Fig. 8. Gibberellin-like substances in terminal embryonic shoots of 20 year old Scots pine trees at the end of June V — vegetative trees, ♀ — female trees, ♂ — male trees

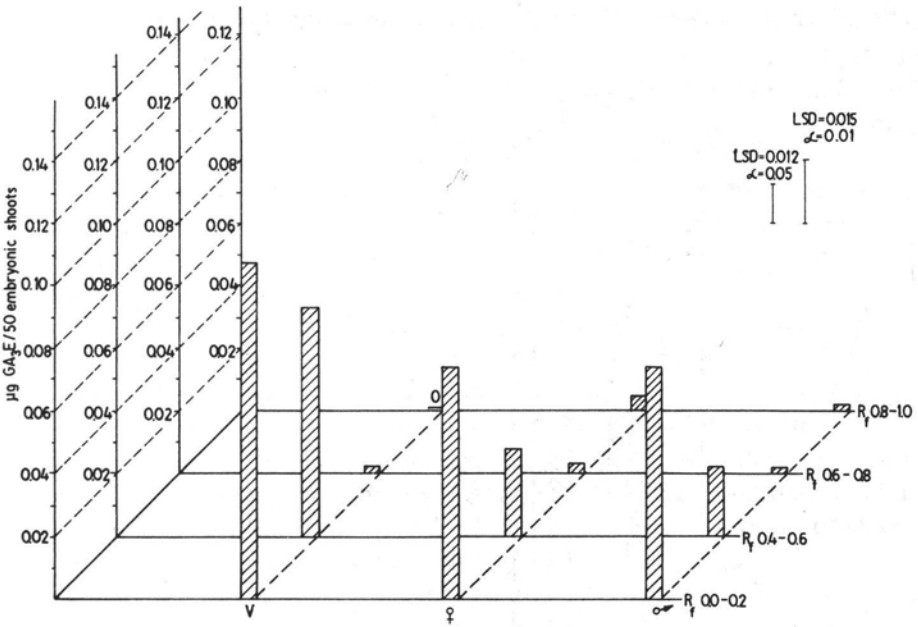

Fig. 9. Gibberellin-like substances in terminal embryonic shoots of 20 year old Scots pine trees at the end of September. V — vegetative trees, ♀ — female trees, ♂ — male trees

Female embryonic shoots of 60 year old trees (Fig. 6) contained, similarly as male embryonic shoots, small amounts of gibberellins. At stage 1 and 2, more of these substances occurred in lateral buds, whereas at stage 3, in terminal buds. The differences between stage 1 and 3 were significant statistically.

In male embryonic shoots at stages 1 and 3, more gibberellins were present in terminal buds, whereas at stage 2, just before the primordia initiation of male inflorescences — in lateral ones. Moreover, at stage 1, a new active zone R_f 0.6-0.8 occurred in male embryonic shoots. Perhaps these gibberellins are responsible for sex differentiation of flowers in the direction of maleness. Similar results were also obtained in earlier investigations (Kopcewicz et al. 1977, 1978, Kulikowska et al. 1978).

The next stage of investigations dealt with young trees, about 20 year old, which began the transition into the generative phase. These trees displayed strong apical dominance covering the whole tree and were characterized by a conical shape. Prevailing here were specimens producing only female cones, but one could also find trees with only male flowers. So, three groups of trees were taken for investigations:

1. vegetative, still not producing flowers;
2. producing only female flowers;
3. producing only male flowers.

From each of the selected groups of trees, material was taken at the following three stages:

1. the stage just before the primordia initiation of male inflorescences (first half of June),

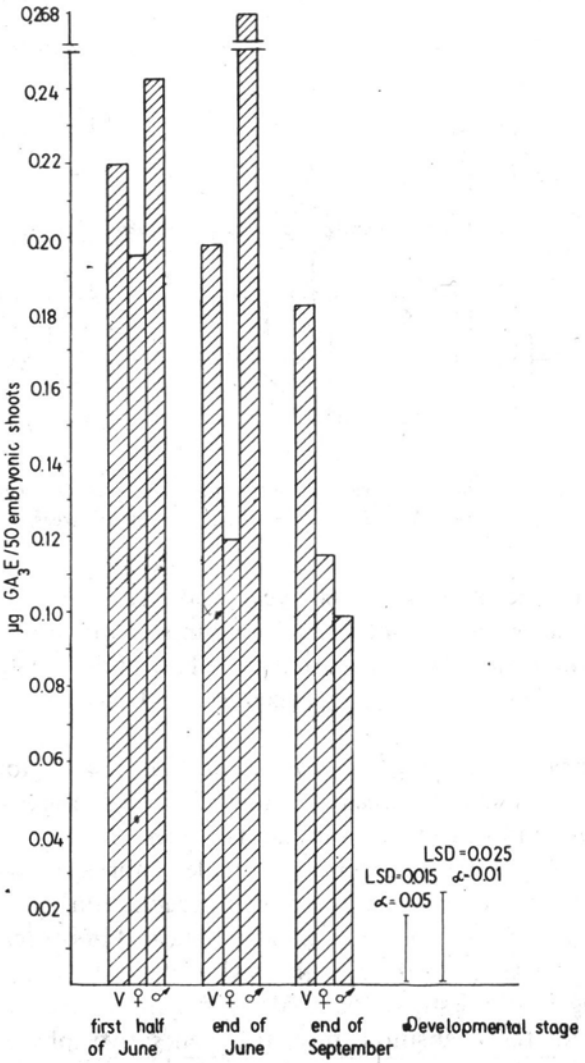

Fig. 10. Total amount of gibberellin-like substances in terminal embryonic shoots of 20 year old Scots pine trees. V — vegetative trees, ♀ — female trees, ♂ — male trees

2. the stage just before the primordia initiation of female cones (the end of June),
3. the stage in which the gradual inhibition of cell division processes

takes place in embryonic shoots as well as the transition of buds to the state of dormancy (the end of September).

Gibberellin-like substances (Figs. 7, 8, 9) from the extracts of all three groups of trees were localized on chromatograms at as many as four active zones: R_f 0.0-0.2, R_f 0.4-0.6, R_f 0.6-0.8 and R_f 0.8-1.0.

Vegetative embryonic shoots contained great amounts of gibberellins, especially at R_f 0.0-0.2 and R_f 0.4-0.6. During three successive stages, the gradual decrease of their content took place, the differences being statistically significant.

Male embryonic shoots were characterized by the highest level of gibberellin-like substances, excluding those at stage 3. At stage 1 (Fig. 7), just before the primordia initiation of male inflorescences, a considerable amount of gibberellins was found at R_f 0.8-1.0, whereas this was not observed at any other stage or any other group of trees. The above suggests the relationship of these substances with sex differentiation of pine flowers in the direction of maleness. At stage 2, one could observe an increase in the content of gibberellins (Fig. 10).

Female embryonic shoots (Fig. 10) contained less gibberellins than vegetative and male embryonic shoots; these differences were statistically significant at stages 1 and 2. At stage 2 (Fig. 8), female shoots contained large amounts of polar gibberellins only, which may be connected with their young age rather than with sex expression of flowers. Similar observations were put forward by Romberger (1976) who showed that the juvenile state was connected with a high concentration of gibberellic acid and more polar gibberellins. So, it may be said that the content of gibberellins, both from the qualitative and quantitative poit of view, in male embryonic shoots differs generally from their content in vegetative and female embryonic shoots. These results find confirmation in earlier investigations on sex expression in pine (Kopcewicz et al. 1977, 1978, Kulikowska et al. 1978). Male embryonic shoots of Scots pine contain the highest level of gibberellins and moreover, a considerable amount of non-polar gibberellin-like substances is also found here.

REFERENCES

Dunberg A., 1974. Occurrence of gibberellin-like substances in Norway spruce (*Picea abies* (L.) Karst.) and their possible relation to growth and flowering. Forest. Suec. 111: 1-62.

Frankland B., Wareing P. F., 1960. Effect of gibberellic acid on hypocotyl growth of lettuce seedlings. Nature 185: 255-256.

Galoch E., Michniewicz M., 1978. Dynamics of endogenous gibberellin-like substances in embryonic shoots of young Scots pines (*Pinus silvestris* L.) at the time of flower primordia initiation. Bull. Acad. Pol. Sci., Ser. Biol. Sci. II, 26: 421-424.

Kopcewicz J., Michniewicz M. Kriesel K., 1967. Dynamics of gibberellin-like substances and growth inhibitors in pine (*Pinus silvestris* L.) and larch (*Larix decidua* Mill.) in relation to the age and season. Bull. Acad. Pol. Sci. 15: 427-433.

Kopcewicz J., Zatorska Z., Kulikowska H., Szcześniak T., 1977. Endogenous growth regulators in embryonic shoots of Scots pine at the time of male and female flower primordia initiation. Acta Soc. Bot. Pol. 46: 119-128.

Kopcewicz J., Zatorska Z., Kulikowska H., Szcześniak T., 1978. Hormonal balance in apical meristems of Scots pine shoots as a very early symptom of flower sex differentiation. Acta Soc. Bot. Pol. 47: 107-114.

Kulikowska H., Kopcewicz J., Zatorska Z., Szcześniak T., 1978. Auxins and gibberellins in embryonic shoots of Scots pine in relation to flower sex differentiation. Acta Soc. Bot. Pol. 47: 403-409.

Romberger J. A., 1976. An appraisal of prospects for research on juvenility in woody perennials. Acta Hortic. 56: 301-317.

Tomaszewski M., 1970. Auxin-gibberellin interactions in apical dominance. Bull. Acad. Polon. Sci., Ser. Sci. Biol. 18: 361-366.

Rola substrancji giberelinopodobnych w różnicowaniu generatywnym pędów embrionalnych sosny zwyczajnej

Streszczenie

Badania prowadzono na pędach embrionalnych bocznych i szczytowych pąków drzew sosny zwyczajnej (*Pinus silvestris* L.) w różnym wieku w kilku okresach sezonu wegetacyjnego. Wyniki wskazują, że istnieje korelacja między zawartością giberelin, a dominacją wierzchołkową u sosny. Więcej giberelin występuje w pąkach szczytowych niż w bocznych. Przejściu sosny od fazy juwenilnej do dojrzałej towarzyszy zmniejszenie zawartości giberelin. Zawiązywaniu kwiatów męskich towarzyszy zwiększenie zawartości endogennych giberelin oraz pojawienie się grupy giberelin niepolarnych. Zawiązywanie kwiatów żeńskich łączy się z obniżeniem poziomu endogennych giberelin.