Seasonal changes of gibberellin-like substances and growth inhibitors in the apical meristems of pine (*Pinus silvestris* L.)*

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Among various publications dealing with growth substances there are but few concerning the changes in the contents of growth regulators during the vegetation of pine trees. These publications concerned on the dynamics of indolic compounds (Zimmermann 1936; Wareing 1951; Brown 1958; Allen 1960; Couvy 1962; Westing 1962; Giertych and Forward 1966; Ogasawara 1966). However, there exists no information as to the role played by gibberellins in the growth processes of pine. The only paper regarding this problem is the publication of Kopcewicz, Michniewicz and Kriesel (1967) about the seasonal changes of gibberellins and inhibitors contents in pine trees of various age.

The present paper is a continuation of those investigations. Its purpose is the examination of the contents of gibberellin-like substances and inhibitors in the meristems of terminal as well as lateral buds during the yearly development cycle of pine.

MATERIAL AND METHODS

The material was taken from 10-year-old pine trees growing in natural stands in the forests of the Forests District Konstantiewo near Golub.

For analyses the excised apical meristems of terminal and lateral buds and shoots growing in the upper parts of trees were used.

The material was taken during the following stages of bud development:

- I. dormant buds — March 2, 1966
- II. swollen buds — April 22, 1966
- III. opening buds — May 5, 1966
- IV. open buds with visible needle primordia — May 26, 1966
- V. active buds with needles extending — June 10, 1966
- VI. active buds with fully extended needles — July 2, 1966
- VII. cessation of all formative activity at apical meristems — September 20, 1966
- VIII. buds gathered in October 15, 1966
- IX. buds gathered in November 17, 1966
- X. buds gathered in December 12, 1966

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XI. buds gathered in January 16, 1967

XII. buds gathered in February 21, 1967.

The excised meristems were ground in a mortar, and subsequently extracted with 80 % methanol for 48 hrs, at $+5^{\circ}\text{C}$. The methanol was evaporated and the remaining water extract was centrifuged. The pure supernatant was evaporated to dryness. The residue was dissolved in 1 ml of absolute methanol and chromatographed. In the case of gibberellins thin-layer chromatography (silica gel, solvent system: benzene—acetic acid 10 : 3 v/v) was used. Eluates were examined for fluorescence. The thin layers were sprayed with 70 % H_2SO_4 , heated at 120°C for 10 min. and examined under UV light.

The growth-inhibiting substances were subjected to paper chromatography (Whatman No. 1, solvent system: n-butanol—acetic acid—water 19 : 1 : 6 v/v).

Each chromatogram contained an equivalent of 100 meristems. The activity of gibberellin-like substances was bioassayed by the lettuce hypocotyl test (Frankland and Wareing 1960), and that of inhibitors by the test of the first leaf of oat (Michniewicz 1961).

The growth inhibition was expressed in activity units. As unit of growth inhibition 10 per cent of the first oat leaf in relation to control was adopted.

The significance of differences was established by estimating LSD at $P = 0.01$. All experiments were repeated four times.

RESULTS

Terminal buds

Gibberellin-like substances were localized in the chromatograms within four different zones (Fig. 1).

The first substance at R_f 0.0—0.2 appeared at the stage of bud swelling and its activity showed a sudden increase at the time of bud opening. The period of intensive shoot elongation was characterized by a gradual decrease of the contents of the same substances followed by a new increase of their activity in September and October. From November on till the end of March no activity of this substance was observed.

The appearance of the second active zone at R_f 0.3—0.5 was connected with an intensive shoot elongation. The same substance was also revealed in the new apical buds during September and October. Later the activity in this area of the chromatogram disappeared.

The third active substance at R_f 0.6—0.7 was found only at the time of bud opening.

The fourth gibberellin-like substances localized in the chromatograms at R_f 0.8—1.0 appeared in the swollen buds. During the bud opening period and at the initial period of strong shoot elongation, the contents of these substances were quite high, but their level decreased markedly during the further phases of development.

Fig. 1. Seasonal changes of gibberellin-like substances in terminal buds of pine

Fig. 2. Seasonal changes of inhibitors in terminal buds of pine

Their appearance was noticed again in September and October. No gibberellins could be detected in winter.

In extracts containing the gibberellin-like compounds also some amounts of substances were found which delayed the growth of the first leaf of oat. These inhibitors, when chromatographed in n-butanol—acetic acid—water 19 : 1 : 6 v/v,

localized at R_f 0.5–0.8 (Fig. 2). No inhibitors, or only small amounts of them, were noticed at the period of intensive growth of shoots. With the beginning of September, however, a systematic accumulation of inhibitors was observed. Their maximum activity occurred at the period of deep dormancy in December. In January a decrease of the inhibitors contents was noted owing to which the activity of these substances remained at the same level throughout the months of February and March. In April, in the period of bud swelling, the level of inhibitors diminished visibly.

Lateral buds

In the extracts of the meristems of lateral buds and shoots the gibberellin-like substances localized in the chromatograms at two active zones (Fig. 3).

The first group of these substances, localizing at R_f 0.0–0.2, appeared in the period of bud opening, reaching its maximum at the initial period of strong

Fig. 3. Seasonal changes of gibberellin-like substances in lateral buds of pine

elongation growth of the shoots. Afterwards the activity of these substances significantly diminished, increasing again in September and October. In November, December, January, February, March and April no traces of these substances could be found.

The second group of gibberellin-like substances localized at R_f 0.8–1.0. They appeared at the moment of bud opening and their amount increased clearly at the period of strong elongation growth of the shoots. However, a decrease of their content followed and finally they disappeared.

The inhibitors, similarly as in the extracts from the meristems of terminal buds and shoots, localized in the chromatograms at R_f 0.5–0.8 (Fig. 4). The low content

Fig. 4. Seasonal changes of inhibitors in lateral buds of pine

Fig. 5. Total activity of gibberellin-like substances in terminal and lateral buds of pine

of inhibitors found at the period of intensive shoot elongation was followed by a period of a high activity of these substances during September, October, November, December, January and February. From March a gradual decrease of the contents of these inhibitors was observed.

Taking into consideration the sum of activities of the different groups of gibberellin-like substances it may be stated that the periodical changes of these

Fig. 6. Total inhibitor activity in terminal and lateral buds of pine

substances in the terminal as well as in the lateral buds of pine were very similar. A high activity of these substances was noticed at the period of opening and at the initial phases of shoot elongation followed by a clear decrease of their content. In September and October the total amount of gibberellin-like substances increased again. In the dormant buds no gibberellin-like substances were found. Generally, the terminal buds and shoots showed a higher level of gibberellins during the particular phases of development than the lateral ones (Fig. 5).

The seasonal changes of substances having the character of inhibitors proved to be also very similar in the terminal as well as in the lateral buds of pine. A rather low level of inhibitors was found in opening buds and at the period of intensive elongation growth of shoots. With the beginning of July an accumulation of growth inhibitors was observed. The maximum peak of these substances was recorded at the period of deep dormancy of buds. Usually, during the particular experimental phases a higher content of inhibitors was recorded in the lateral buds and shoots than in the apical ones (Fig. 6).

DISCUSSION

The previous paper dealt with some changes in the content of gibberellin-like substances during the vegetation period of 2, 10, 40, 90 and 140 years old pine trees (Kopcewicz, Michniewicz and Kriesel 1967). For experiments the whole buds or shoots of pine were sampled. The results of those experiments showed that resting buds have no gibberellins but they contain large amounts of inhibitors. In the course of bud development some gibberellin-like substances appeared and the concentration of inhibitors gradually decreased.

This paper gives a survey of the seasonal changes taking place in the contents of growth regulators in the meristems of terminal and lateral buds during the annual development cycle of 10-years-old pine trees.

It has been confirmed that gibberellins do not occur in the resting buds of pine. From the beginning of November throughout December, January, February and March no gibberellins were found in the meristems, whereas from July on a gradual increase of the activity of growth inhibitors was observed with a peak at the period of deep dormancy of buds. In spring, a significant decrease of the content of inhibitors was noticed followed by the appearance of gibberellin-like substances. A high concentration of gibberellins was found at the time of bud opening and at the beginning of intensive shoot growth. A similar dependence was found in the earlier experiments with pine (Kopcewicz, Michniewicz and Kriesel 1967).

Such a dependence has been also found by Kamieńska (1966) who experimented with poplar trees.

At later stages the activity of gibberellins diminished and it was followed by an increasing concentration of inhibitors.

Quite interesting seems to be the accumulation of gibberellins in September. It may be assumed that the increased level of gibberellins at this period could possibly be due to the intensified cell division connected with the strong development of the lateral organs. As reported by Giertych (1966), pine buds contain at that time also a high level of auxins.

This paper is the first report including some comparative studies concerning the dynamics of gibberellins in the developing terminal and lateral buds of pine. It has been established that, although the dynamics of gibberellins and inhibitors are rather similar, terminal buds and shoots contain, as a rule, more gibberellins

and less inhibitors than lateral buds and shoots. The differences between terminal and lateral buds concerned not only quantitative but also some qualitative changes in the content of gibberellin-like substances. At the period of intensified biosynthesis of these compounds as many as four groups of gibberellin-like substances were found in the terminal shoots, and only two groups in the lateral ones. Similar data on the occurrence of various gibberellins in pine were presented in the previous papers (Michniewicz and Kopcewicz 1966; Kopcewicz, Michniewicz and Kriesel 1967; Kopcewicz 1968 a, b).

A closer physico-chemical and physiological description of properties of the gibberellin-like substances occurring in pine is given by Kopcewicz (1968 c).

SUMMARY

1. The dynamics of gibberellin-like substances and inhibitors during the annual development of pine buds was approximately the same in the terminal buds and in the lateral ones.
2. Terminal buds and shoots of pine contain larger amounts of gibberellins and smaller amounts of growth inhibitors than the lateral buds and shoots.
3. The dormant buds of pine are characterized by a lack of gibberellins and a high level of growth inhibitors.
4. The breaking of bud dormancy is connected with the beginning of gibberellin production and a decrease of the level of inhibitors.
5. The opening of buds and the initial phases of intensive shoot growth are accompanied by a high level of gibberellin-like substances and a low level of growth inhibitors.
6. The completion of shoot elongation coincided with a decreased amount of gibberellins and the appearance and accumulation of growth inhibitors.

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Sezonowe zmiany w zawartości substancji giberelino-podobnych i inhibitorów w wierzchołkowych merystemach sosny (Pinus silvestris L.)

Streszczenie

Przebadano sezonowe zmiany w zawartości substancji giberelinopodobnych i inhibitorów wzrostu w rocznym cyklu rozwojowym szczytowych i bocznych pąków i pędów sosny.

Do badań pobierano wyizolowane merystemy wierzchołkowe, stosując ekstrakcję 80% alkoholem metylowym oraz chromatografię bibulową i cienkowarstwową.

Aktywności substancji wzrostowych oznaczano za pomocą testów biologicznych: hypocotyli sałaty (gibereliny) oraz pierwszego liścia owsa (inhibitory).

Na podstawie osiągniętych wyników wyciągnięto następujące wnioski:

1. Dynamika substancji giberelinopodobnych i inhibitorów w czasie rocznego rozwoju pąków sosny przebiega podobnie zarówno u pąków szczytowych, jak i bocznych.
2. Pąki i pędy szczytowe sosny zawierają więcej giberelin a mniej inhibitorów wzrostu aniżeli pąki i pędy boczne.
3. Pąki spoczynkowe sosny charakteryzują się brakiem giberelin i wysokim poziomem inhibitorów wzrostu.
4. Przerwanie okresu spoczynku wiąże się z rozpoczęciem produkcji giberelin i obniżeniem poziomu inhibitorów.
5. Otwieraniu się pąków i początkowym fazom intensywnego wzrostu pędów towarzyszy wysoki poziom substancji giberelinopodobnych i niski poziom inhibitorów wzrostu.
6. Końcowy etap wzrostu elongacyjnego pędów łączy się z obniżeniem się poziomu giberelin i zwiększeniem się ilości inhibitorów.