Effect of gravity on the distribution of growth inhibitors in apple tree

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Abstract

The aim of this work was to find, if there is an asymmetrical distribution of growth inhibitors in apple trees being kept in horizontal position for a short period of time. Two inhibitors were found in the extract of bark and young shoots of investigated trees. These inhibitors are identical with phloridzin and abscisic acid. The content of phloridzin was higher in the samples taken from the upper side than from the lower ones. The results showed lack of asymmetry in ABA distribution.

INTRODUCTION

The influence of gravity on plant morphogenesis is known as gravimorphism (Wareing and Nasr 1958). Under the effect of gravity, the growth of young shoots on horizontally placed trees becomes asymmetrical and the shoots growing on the lower (under) side of such trees are weaker than those on the upper side (Borkowska and Jankiewicz, 1972).

Several authors paid attention to the role of growth regulators in gravimorphism. Their results are however, contradictory.

Mullins (1965) believed, that the inhibitors or stimulators in supraoptimal concentration accumulate on the lower side of horizontally placed tree and inhibit the growth of the lower shoots. Smith and Wareing (1964), however, did not find differences in the content of growth regulators in "extra-cambial" tissues isolated from upper and lower side of willow stem. Later on, Leach and Wareing (1967) determined that the concentration of endogenous inhibitors was greater in the lower part of a poplar tree growing horizontally. They defined these inhibitors as "beta inhibitor" and catechol. On the contrary, how-

ever, Nečesany (1958) have found higher content of the growth inhibiting substances in the upper bark of poplar tree.

The aim of this work was to find, if there is an asymmetrical distribution of growth inhibitors in apple trees growing in a horizontal position for a short period of time.

MATERIAL AND METHODS

Young apple trees (Cortland variety) were bent to the horizontal position for 6 or 12 days at bud bursting time. Then samples of the bark and young lateral shoots were collected separately from upper and lower sides. They were frozen immediately in dry ice and stored in a freezer in -15° to -20°C .

Four replicates of plant material were used. Material for one replicate was collected from 5 trees.

Samples 10 g of young shoots and of the bark were homogenized in 80% ethanol. The homogenate was filtered and plant residue discarded. The ethanol extract was then evaporated in vacuum in $45-50^{\circ}\text{C}$. until the aqueous phase remained, which was acidified to pH 2-3 and extracted with ethyl ether 3 times. The acidic ether fraction was then evaporated to dryness, redissolved in small volume of ethanol and chromatographed first on Whatman No. 3 paper and next on Whatman No. 1 in isopropanol : ammonia — water (10:1:1) — in both cases. The wheat coleoptile bioassay was used for detection of the inhibitor zones on the chromatogram. These zones were then eluted with 96% ethanol and rechromatographed using TL chromatography method.

For the separation of compounds located in the first inhibitory zone (R_f 0.36 - 0.45) cellulose powder MN 300 was used. Plates were developed in 2% formic acid. Synthetic phloridzin was used as a standard.

Substances located in the second zone (R_f 0.65 - 0.78) on the paper, were further separated on plates coated with silica gel GF 254 (Merk). Plates were chromatographed first in the solvent benzen - ethyl acetate - acetic acid (80:20:5) and then after elution and evaporation, active substances were again separated in a solvent benzen - acetone - acetic acid (70:30:1). Synthetic abscisic acid (ABA) was used as a standard.

The activity of both inhibitors present in samples taken up from the upper and the lower side of the tree was determined by wheat coleoptile test using several dilutions.

RESULTS

Two zones of marked inhibition in wheat coleoptile bioassay were found on paper chromatograms after separation of extracts of bark and

Fig. 1. The content of phloridzin in the trees kept in horizontal position for 12 days

young shoots. R_f values of these inhibitors in isopropanol - ammonia - water (10:1:1) were: 0.36 - 0.45 and 0.64 - 0.78.

The inhibitor present at R_f 0.36 - 0.45 was co-chromatographed with synthetic phloridzin in several solvents on P.C. and TLC. Its R_f values were always the same as those of phloridzin. While treated with diazotized benzidin it gave the same as phloridzin colour reaction. The inhibitor gave also identical with phloridzin colour of fluorescence under UV- light, as well in ammonia vapour as without it.

The inhibitor from the second zone (R_f 0.64 - 0.78) was eluted from

Fig. 2. The content of ABA in the trees kept in horizontal position for 12 days

paper chromatogram and further purified by TLC on silica gel GF 254. Its R_f was the same as that of synthetic ABA.

These results and the experience from previous work done in our laboratory on identification of phloridzin (Pieniążek 1962) and ABA (Pieniążek, Rudnicki 1967) suggest that the two isolated inhibitors are identical with phloridzin and ABA respectively.

Biological activity of phloridzin isolated from fresh young shoots was lower than that from the bark, as shown by dilution method. Dilution $\times/8$ of the bark extract gave 90-95% of inhibition of coleoptiles elongation, whereas that dilution of young shoot extract gave only 20-34% of inhibition (Fig. 1).

The results presented in Fig. 1 show that the level of phloridzin was higher in the bark and young shoots taken up from the upper side of the tree than from the lower.

The activity of ABA was similar in extracts from the bark and from young shoots taken from upper and lower side of the tree (Fig. 2).

The length of the period of growth in horizontal position had not a visible influence on the content of both inhibitors.

DISCUSSION

It was found earlier (Borkowska 1968), that apple trees kept in horizontal position for 3-5 days or longer showed marked inhibition of shoot growth on their lower side.

This inhibition was very marked also in trees growing horizontally in complete darkness (Borkowska and Jankiewicz, 1972).

The samples for analyses were therefore taken from the trees kept in horizontal position for 6 and 12 days. After 6 days in horizontal position the difference in development of upper and lower buds was slightly visible, but after 12 days it became already distinct.

Two inhibitors were found in the extract of bark and shoots of investigated trees.

It was rather surprising that phloridzin content was higher in the bark and the shoots taken from the upper side of the tree, than in the lower ones.

It is difficult to define the cause of this phenomenon. It is however, doubtful that phloridzin acts as an inhibitor in apple tree. Kamiński and Pieniążek (1968) have found synergistic stimulatory effect of phloridzin with gibberellin and cytokinins in apple seed germination.

Presented results show lack of asymmetry in ABA distribution in horizontally placed trees. They are conformable to those of Smith and Wareing (1964). These authors have found equal level of unidentified inhibitor in the bark from upper and lower side of willow trees.

It may be postulated, on the basis of these results, that investigated growth inhibitors probably do not play an important role in the mechanism of gravimorphism.

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Wpływ grawitacji na rozmieszczenie inhibitorów wzrostu w drzewkach jabłoni

Streszczenie

Celem niniejszej pracy było zbadanie rozmieszczenia inhibitorów wzrostu w młodych drzewkach jabłoni rosnących w pozycji poziomej w ciągu krótkiego okresu czasu.

W ekstraktach z łyka i młodych pędów lub rozwijających się pąków zostały znalezione 2 inhibitory. Wartości R_f tych inhibitorów w solwencie izopropanol - amoniak - woda (10:1:1) wynosiły: 0,36 - 0,45 i 0,64 - 0,78. Otrzymane wyniki wskazują, że inhibitory te są identyczne z florydzyną i kwasem abscysynowym.

Zawartość florydżyny była wyższa w łyku i liściach wziętych ze strony górnej drzewka poziomego, niż ze strony dolnej.

Zawartość kwasu abscysynowego była jednakowa po górnej i dolnej stronie drzewka poziomego.