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PHENOLICS FROM LARIX NEEDLES. VI. EFFECT OF SHORT-DAY TREATMENT

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SUMMARY

A short-day (SD) photoperiod of 3 to 7 days was critical for delay in budding of defoliated seedlings. Both growth-stimulating and growth-inhibiting substances were present in *Larix* needle extracts. SD treatment induced a decrease of stimulation and an increase in inhibiting activity. The concentration of one of the main flavonoids, a vitexin derivative, was significantly decreased in SD needle extracts.

1. INTRODUCTION

Larch is very sensitive to SD photoperiods. Four short days, followed by a long-day (LD) period sufficed for an indication of partial dormancy, visible after 3 weeks (VAN DER VEEN & MEIJER 1958). Dormancy has often been causally related to the production of growth inhibitors (WAREING 1954) or to a decrease in growth-promoting substances (WAREING & SAUNDERS 1971). WODZICKI (1964) demonstrated a significant change in both growth-inhibiting and growth-promoting substances in the apical and cortical tissues of *Larix decidua* as a result of SD treatment.

In several cases accumulation of growth inhibiting activity has been found to be due to phenolics, for example to naringenin in resting peach buds (HENDERSHOTT & WALKER 1959), quercetin and quercitrin in willow shoots (KEFELI & TURETSKAYA 1965, 1966) and gallic acid in *Kalanchoe* (PRYCE 1972). On the other hand, the importance of light in the regulation of flavonoid biosynthesis in higher plants has been well established (SIEGELMAN 1964).

Our interest in *Larix* phenolics (NIEMANN 1969, 1971, 1972, 1973; NIEMANN & BEKOOY 1971) and their physiological significance led us to look into possible changes in the phenolic composition as a result of an SD photoperiod.

2. MATERIAL AND METHODS

Seedlings of *Larix leptolepis* (Sieb. et Zucc.) Gord. were kept in the greenhouse under LD conditions. Plants 17-22 months old were moved before use to a climate chamber, temperature 20 °C, with either LD (18 hours light) or SD (8 hours light).

Needles were picked and extracted with cold ethanol or with acetone. The extracts were concentrated, extracted with light petrol and further separated by one- or two-dimensional paper chromatography. Eluates of the chromatograms

were tested in an *Avena* coleoptile section test according to RIETSEMA (1950).

The remaining leafless seedlings were either kept under the same conditions or transferred to the LD cell; the time of budding of the first bud and that of more than two and more than ten, were noted.

3. RESULTS AND DISCUSSION

Buds of defoliated plants kept under SD after picking the needles remained dormant*. This points to a perception of long nights by the resting buds, quite in agreement with WAREING's results (1954) with *Betula*. Under LD, budding of defoliated plants occurred in all cases. A previous SD treatment, before picking the needles, however, significantly influenced the time it took for the first bud to break. This was especially apparent with a SD period of 3 to 7 days. Without previous SD the first bud always broke within ten days after defoliation, with an average of 7.7 days for 12 plants. Previous SD, from 3 up to 7 days, led to increasing budding time with a maximum of about 2 to 3 weeks after defoliation. Lengthening of the SD period from 7 days up to 121 days did not lead to any further increase, the first bud always broke within two or three weeks.

Real dormancy, which can only be broken by chilling or hormone treatment, was never found. In this aspect the seedlings used differ from mature plants grown under natural conditions. Branches from the latter, when collected in December, could not be brought to budding under similar conditions as the seedlings. However, when taken from the same tree in January, budding occurred in about 8 days, independent of LD or SD, although the needles formed under SD soon died.

The ethanolic extracts of LD needles inhibited the growth of *Avena* coleoptile sections. However, after banding on paper with 15% acetic acid, both a zone with a stimulating effect and one with an inhibiting effect were found. Preliminary tests also indicate an increase of the inhibiting activity and a decrease of the stimulating activity of SD extracts (35 short days) as compared with corresponding LD zones.

A marked difference was observed when LD and SD extracts were compared for flavonoids and other phenolics. Although in general LD and SD extracts gave the same pattern, for one spot on the chromatograms the concentration was significantly decreased in SD needles. After 21 to 35 short days this flavonoid usually was totally absent on the chromatograms. This compound also formed the main flavonoid in the LD inhibiting zone (coleoptile section test).

A small quantity of the compound was isolated in solution; it had the properties of a (glucosyl) glycoside of vitexin. According to the UV spectral data all flavonoid hydroxyls were free. Acid hydrolysis gave vitexin and the sugars glucose and xylose. Thus the compound could be a vitexin derivative with both

* With two exceptions: after an SD period of 20 days and 6 months respectively on two different plants some buds broke, giving incomplete leaves, which soon crumpled and died.

glucose and xylose attached to the C-8-glucose. It is much more likely, however, that it is a mixture of glucosylvitexin and xylosylvitexin. $R_f$values (6 on paper, 3 on thin layer) were identical with those of xylosylvitexin.

The question remains whether there may be any connection between the induced dormancy, the stimulated inhibiting activity in the coleoptile section test, and the disappearance of the vitexin derivative, all as a result of an SD photoperiod. The time necessary for a first, clearly visible, induction of postponed budding was about 10 days (3 SD followed by 7 LD or SD). The time needed for the decrease of the vitexin derivative was 14 to 21 SD. This difference might render a causal relationship between both phenomena less probable. However, further research in this connection is necessary.

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