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PHENOLICS FROM LARIX NEEDLES. XII. SEASONAL VARIATION OF MAIN FLAVONOIDS IN LEAVES OF L. LEPTOLEPIS

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SUMMARY

The metabolic fate of six flavonoids during development and growth of larch needles was investigated. One flavonoid, dihydroquercetin, appeared to be a special bud-flavonoid. Four compounds were isolated both from buds and leaves, a maximum concentration was found in developing young leaves. A sixth flavonoid, kaempferol-3-(*p*-coumarylglucoside), was nearly absent in the buds, its highest concentration in the leaves occurred about two months later than that of the other compounds. Only one compound, naringenin-7-glucoside, tended to reaccumulate in late summer.

1. INTRODUCTION

In *Larix* needles a rich array of flavonoids has been found with a very complete hydroxylation/methoxylation pattern (NIEMANN 1974, 1975b). The same flavonoids were found in needles of all larch species investigated (*L. laricina* – NIEMANN & BEKOOY 1971, NIEMANN 1972; *L. leptolepis* – NIEMANN 1973, 1974; *L. sibirica* – MEDVEDEVA et al. 1972a, b, c, 1973, 1974a; *L. dahurica* – TJUKAVKINA et al. 1975; *L. decidua* – NIEMANN 1975a; *L. gmelinii* – NIEMANN 1975b). Only quantitative differences were present, *L. sibirica* needles, for example, were found rich in kaempferol-3-glucoside, *L. decidua* in quercetin derivatives, and *L. dahurica* in myricetin-3-glucoside. These data, however, only represent the flavonoid composition in needles collected at one time, mainly in summer. It has been known for some years that in different plants the flavonoid composition is not constant, but may be subjected to significant variation (TISSUT & EGGER 1972, STAUDE & REZNIK 1973b). Specially during periods of active growth and/or differentiation considerable changes in flavonoid composition and concentration may occur (STAFFORD 1969, WEISSENBÖCK & REZNIK 1970, STAUDE & REZNIK 1973b). Thus, in addition to genetic variation, physiological factors may in part be responsible for the quantitative differences found for larch flavonoids. More knowledge on the seasonal variation of larch flavonoids became desirable.

Possibly this holds even more in connection with the special place of *Larix* among the Pinaceae. It is the only genus in which all leaves are shed in autumn.

In its leaf flavonoids larch also appears rather outstanding in having methylgalloyl- and syringyl-structures (Compare: *Picea* – DITTRICH & KANDLER 1971; *Abies* – MEDVEDEVA et al. 1974b; *Pseudolarix* – NIEMANN 1975c).

Of the larch species *L. leptolepis* was chosen for investigation for its intermediate flavonoid composition in August needles. Main flavonoids identified in this species were: kaempferol-3-glucoside, kaempferol-3-(*p*-coumarylglucoside), and isorhamnetin-3-glucoside. Lower concentrations were found of quercetin-3-glucoside, 3′-methylmyricetin-3-glucoside, syringetin-3-glucoside and -3-rutinoside, vitexin and its (glucosyl) xyloside and glucoside, dihydroquercetin and its 3-glucoside*. Low to very low concentrations were present of kaempferol-3-rutinoside* and -3-arabinoside, quercetin-3-arabinoside*, isorhamnetin-3-rutinoside and -3-arabinoside, 3′-methylmyricetin-3-arabinoside, and syringetin-3-arabinoside (NIEMANN 1973, 1974 and unpublished results).

2. MATERIAL AND METHODS

Between April 1973 and April 1974 needles of *Larix leptolepis* (Sieb. et Zucc.) Gord., growing on short shoots, were collected from a marked tree at Austerlitz, The Netherlands. The needles were frozen to dryness and samples of ½ to 1 g were extracted according to the procedure of TISSUT & EGGER (1972). Ether and butanol fractions were separated by two- and/or one-dimensional paper chromatography. For a quantitative investigation compounds with reasonable concentration and/or significant variation were of interest. Originally, four compounds were chosen: kaempferol-3-glucoside (KG), kaempferol-3-(*p*-coumarylglucoside) (KCG), isorhamnetin-3-glucoside (IG), and a hitherto unidentified flavanone. This flavanone was later tentatively identified as naringenin-7-glucoside (NG). The compounds were eluted and relative concentrations were measured on an Optica CF 4R spectrophotometer. For the flavonols the absorbance of the UV long wave absorption maximum was used, for flavanones that of the short wave one. Sometimes repeated chromatography was required for good separation. All compounds were rechromatographed (original and hydrolysis products) for identification.

From this procedure the IG spot, which contained almost pure IG in the August needles, appeared to consist of a mixture of IG and quercetin-3-glucoside (QG). The ratio QG/IG varied, with high QG in spring to neglectable QG values in August.

In a second series, collected from February to June 1975, in addition to KG, KCG, IG and NG, QG and dihydroquercetin (DHQ) were measured as well. In this series 2 g fresh needles were extracted instead of 1 g dry ones.

* Not published before in *L. leptolepis*

3. RESULTS AND DISCUSSION

Data for the seasonal variation of KG, KCG, IG (+QG) and NG in 1973–74 are shown in *fig. 1*. For most compounds the more active metabolic period is found between the bud stage and leaf development till about June. In general, an initial raise in concentration from March to May is followed by a decline and a more stabilized situation throughout the summer. Only for the flavanone (NG, graph d) more variable concentrations were found. Graph b suggests that KCG synthesis starts later or more slowly than that of the other glycosides.

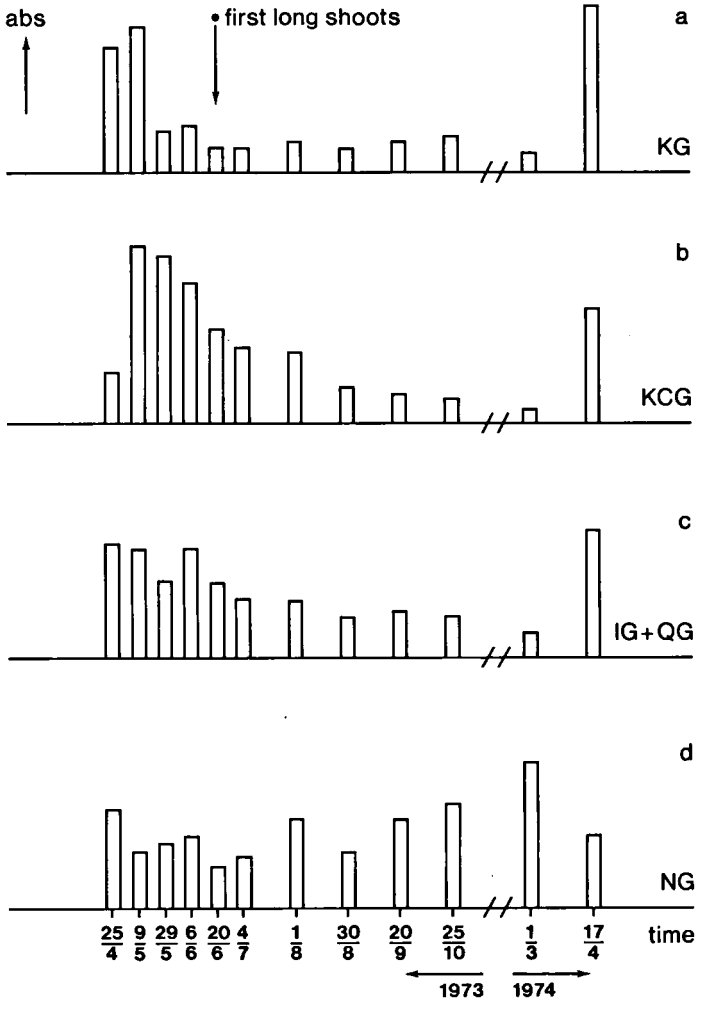

Fig. 1. Changes in flavonoid concentration during leaf development in 1973–1974 per g dry weight.

Possibly its synthesis is dependent on an initial concentration of KG.

Relative concentations are given, based on dry weight. From *fig. 2b* it can be seen that comparison on a fresh weight basis gives no significant changes, except in case of the bud stage. Here, on a fresh weight basis the level for KG, KCG and IG + QG increases to that of summertime values. For NG, however, the already high level becomes much more pronounced.

More information may be obtained by comparing the concentration per needle. *Fig. 2a* gives the average needle weight and *fig. 3* the derived values of *fig. 1*. In general, the maximum concentrations appear somewhat more pronounced and shifted to a later period. The 3-glycosides have a tendency to go from an initial rise in spring to a more stable, lower level in summer. NG, however, appears to build up a higher concentration in summertime.

The most active period for the investigated compounds seems between the initial bud phase and the moment at which the growth of long shoots starts. In spring 1975 this period was reinvestigated with weekly intervals. The results are combined in *fig. 4*. For the buds of February 27 difference was made between the closed swollen buds (shaded blocks) and the burst ones (open blocks) on the same branches. For three compounds, KG, QG and NG the maximum concentration is reached between the middle of March and the middle of April.

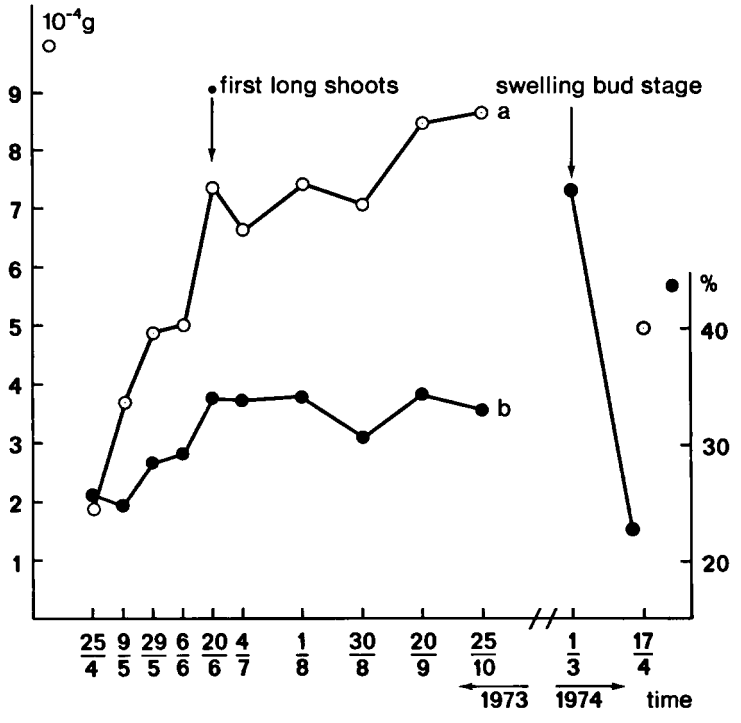

Fig. 2. Average dry needle weight (a) and dry weight given as a percentage of fresh weight (b).

Before the growth of long shoots starts this concentration declines to a much lower level. IG is somewhat different by having some indication of a maximum at the same time, which, however, is not followed by a decline.

A quite different behaviour is shown by KCG and DHQ. The latter seems an exclusive bud flavonoid as it could not be detected in the needles with the method used. (As shown previously (NIEMANN 1974) DHQ could be isolated in low concentration from much greater badges of August needles.) Synthesis of KCG starts later than that of the other compounds. Again the rise in KCG at the time the concentration of KG drops suggests a possible use of KG for KCG synthesis. On the other hand the similarity in graphs a, c, and to a lesser extent in d, tends to make this possibility less probable.

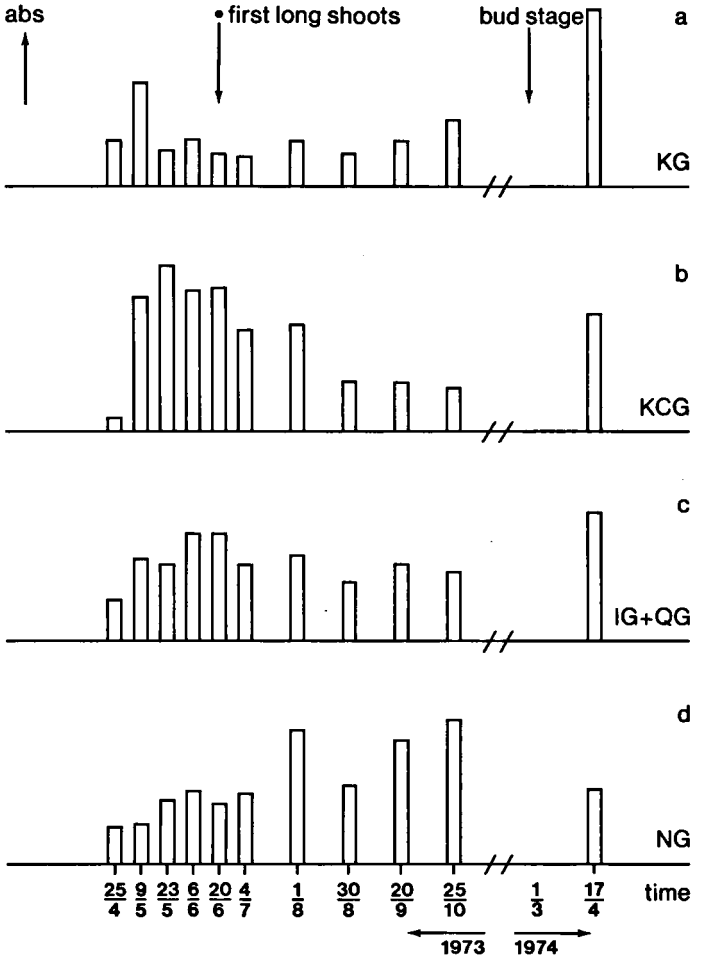

Fig. 3. Changes in flavonoid concentration during leaf development in 1973–1974 per needle.

One item in most curves, a depression in the period between March 13 and April 10, instead of a more expected gradual course, may be due to weather influences. After a normal begin of March, from the 16th onwards an abnormal cold period with night frost and snow days occurred lasting till April 11. A

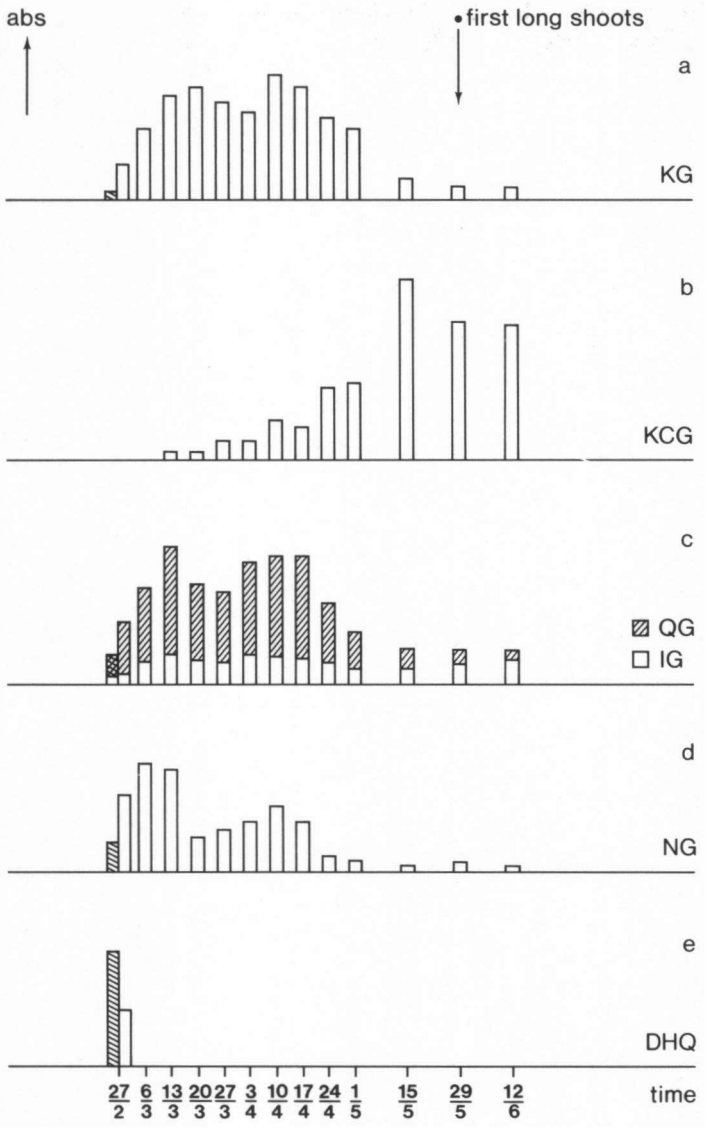

Fig. 4. Changes in flavonoid concentration during bud and leaf development in 1975, per g fresh weight. Shaded blocks at 27.2 stand for closed buds, open blocks at the same date for burst ones on the same branches.

similar depression can be seen in the curves representing the average needle weight (*fig. 5*). Combination of the values given in *figs. 4* and *5a* gives the relative concentration per needle, represented in *fig. 6*. Especially in graphs a and b a sudden drop of KG and the increase of KCG between the first and fifteenth of May becomes much more pronounced.

In addition to the investigated flavonoids two other phenolic compounds were present in the needle extracts in relatively high concentration. Since both phenolics appeared to be *p*-coumaric acid derivatives a possible relation with KCG synthesis can not be excluded. Therefore, these compounds were also investigated. The first *p*-coumaric acid derivative was a sugar ester, for which

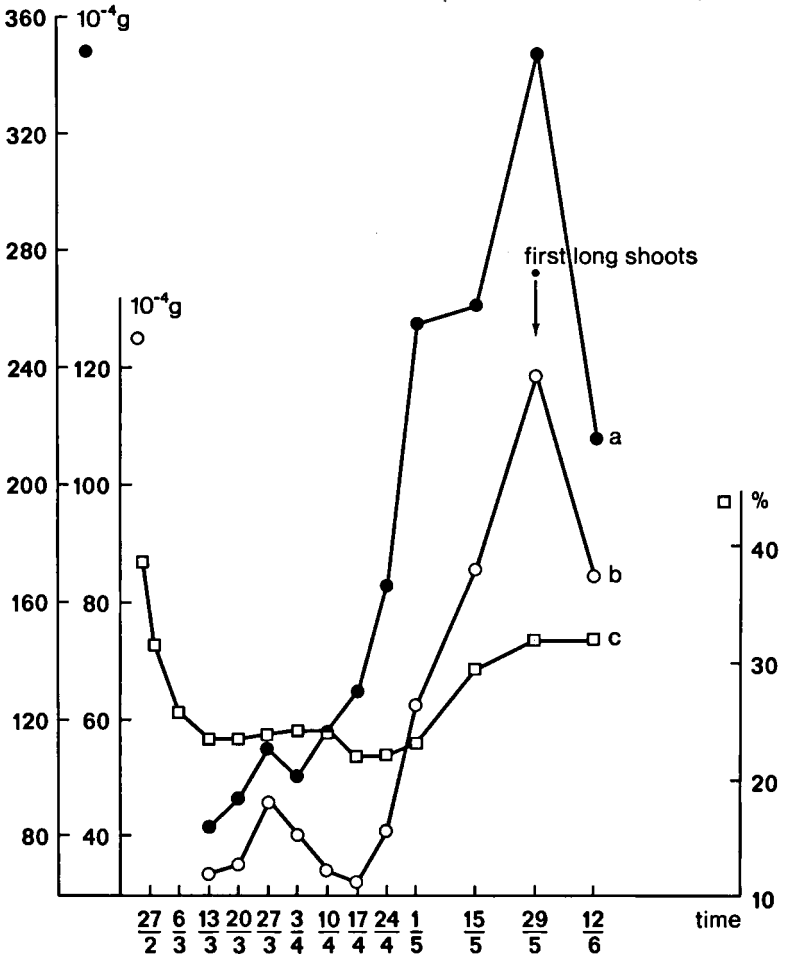

Fig. 5. Average fresh (a) and dry (b) needle weight; and dry weight (c) given as a percentage of fresh weight.

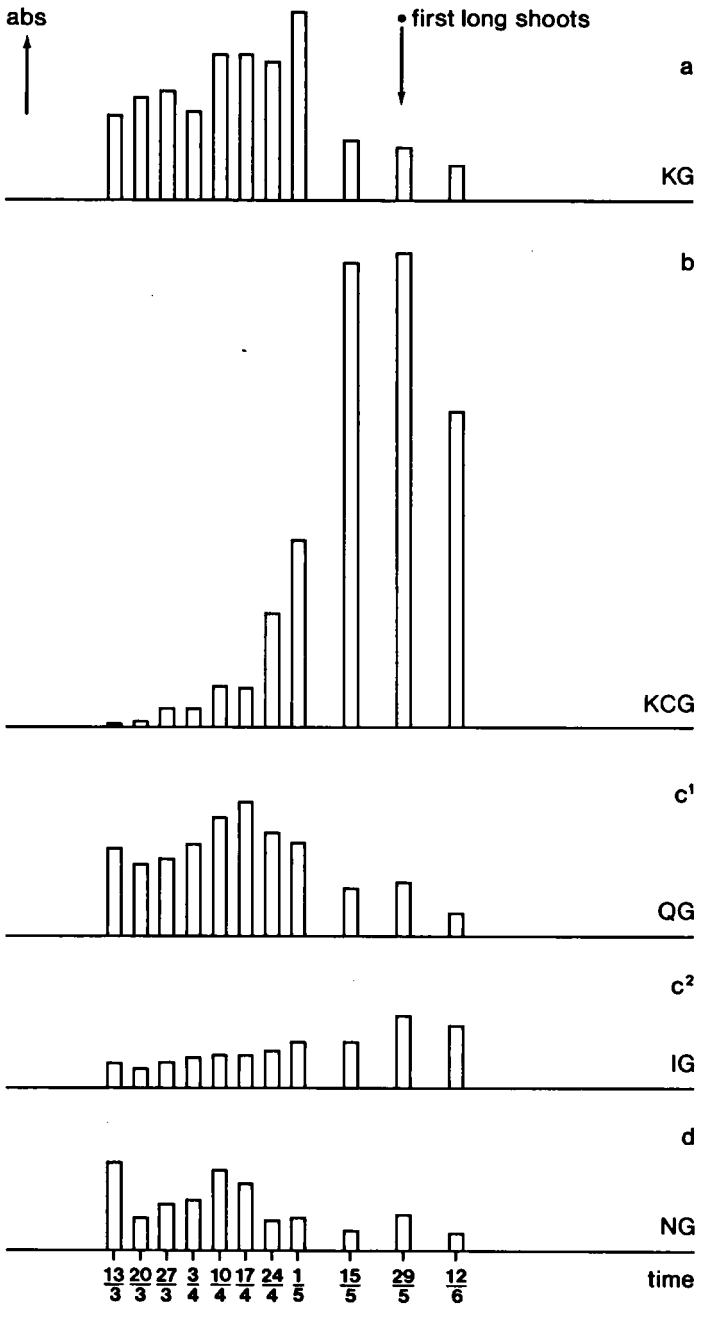

Fig. 6. Changes in flavonoid concentration during development of the young leaf in 1975, per needle.

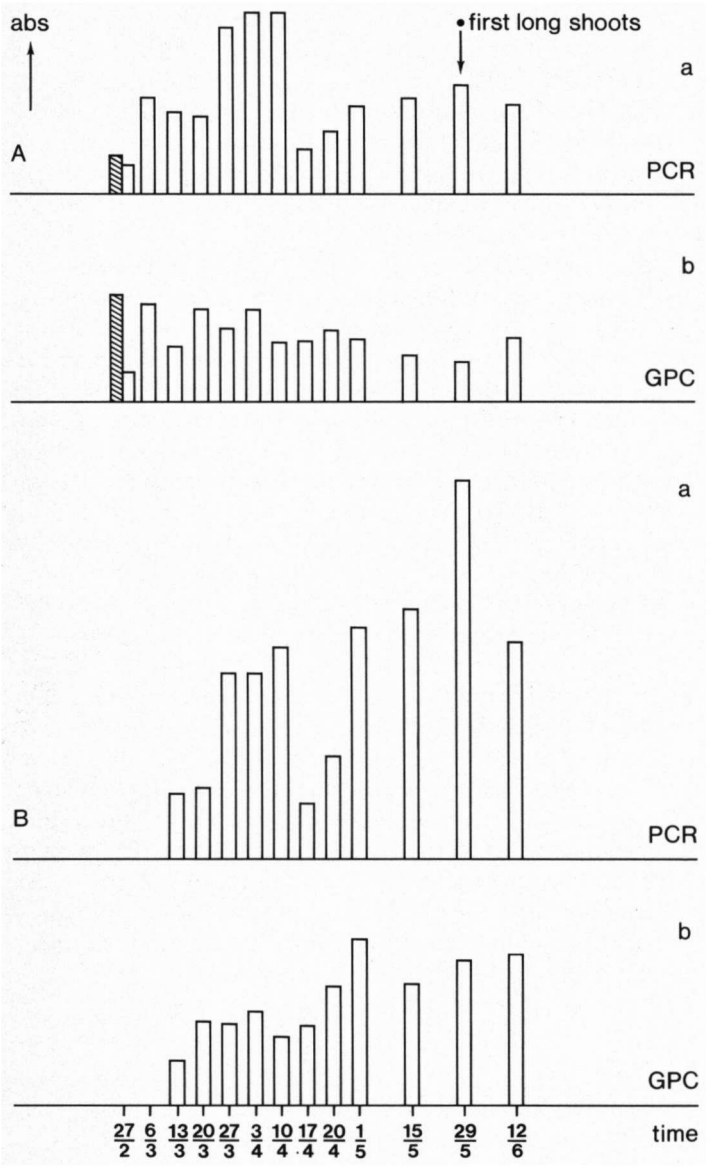

Fig. 7. Changes in concentration of *p*-coumarylrutinose (PCR) and of glucosyl-*p*-coumaric acid (GPC) per g fresh weight (A), and per needle (B). For shaded blocks see *fig. 4*.

all data point to *p*-coumaroylrutinose (PCR). The second compound was not completely purified before UV measurement since it consisted mainly of glucosyl-*p*-coumaric acid (GPC), mixed with traces of the difficultly removable glucoside of ferulic acid. The test results are summarized in *fig. 7*. GPC remains on a more or less constant level. In the ester graph, however, a sudden drop in concentration was found at a rather early date (17/4), after which a fast recovery occurred. Again the question arises whether a transesterification between PCR and KG is responsible for KCG synthesis.

The physiological role of flavonoids is still vague. In some cases a possible function as cofactor or inhibitor of indoleacetic acid oxidase has been reported (FURUYA et al. 1962; NITSCH & NITSCH 1962). In other cases growth-inhibiting flavonoids have been isolated from dormant plant material, like naringenin from dormant peach buds (HENDERSHOTT & WALKER 1959; PHILLIPS 1961) and quercetin derivatives from willow shoots (KEFELI & TURETSKAYA 1965, 1966). In the latter work accumulation of the flavonoids in late summer and autumn was shown. Such an accumulation seems rather uncommon. In general, flavonoids in full-grown leaves stay at a more or less constant level (TISSUT & EGGER 1972) or they diminish at the end of the vegetative period like in *Corylus* leaves (STAUDE & REZNIK 1973b). Most *Larix* flavonoids follow the latter pattern, with the exception of naringenin-7-glucoside (and possibly isorhamnetin-3-glucoside). NG, after a previous decline in May-June tends to reaccumulate in summer.

A difference between flavonoid composition in buds or young leaves and in full-grown leaves has been found in three out of six trees investigated (TISSUT & EGGER 1972). Such an aspect is especially obvious in *Corylus* buds and leaves (STAUDE & REZNIK 1973a). The three main bud flavonoids were not present in the leaves. One of these compounds is a kaempferol-3-(*p*-coumarylglucoside) which may be identical with *Larix* KCG. The latter, however, is nearly absent in buds and its accumulation in young leaves only starts about two months after that of the other leaf-flavonoids. Of the compounds investigated only dihydroquercetin seems a real bud flavonoid with a rapid break-down at the moment of bud burst.

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