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Seasonal Variation of Pigments in Needles A Study of Scots Pine and Norway Spruce Seedlings Grown under Different Nursery Conditions

Årstidsvariation av pigment i barr från plantor av tall och gran odlade under skilda plantskolebetingelser

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Abstract

ODC 164.5: 161(485)[—174.7 Pinus sylvestris + 174.7 Picea abies]

The influence of growing conditions upon the pigmentation in needles from seedlings of Scots pine and Norway spruce has been studied. The plant material was grown either in the open or in plastic greenhouses. The greenhouses were covered with plastic during the vegetation periods from May to August, and during the rest of the year the 'greenhouse' grown seedlings were exposed to outdoor conditions.

A pronounced seasonal variation of both chlorophyll and carotenoids was found. The content of pigments was influenced also by growing conditions, plant age, needle age and origin of the seed source.

The new technique of growing coniferous seedlings in greenhouses during the vegetation period does not seem to have any harmful effect on the seedlings, as far as the pigmentation of the needles is concerned.

Since the seasonal discoloration of needles in many cases can resemble changes caused by nutrient deficiencies, caution should be exercised whenever the needle color is used as an index of the physiological state of nursery stocks.

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Content

1 Introduction

During the last decade many forest nurseries have started to grow their seedlings in plastic greenhouses under partially controlled environmental conditions. During the vegetation period the seedlings are grown in greenhouses but in the late summer or autumn the plastic cover is removed and the plants are then exposed to outdoor conditions until the beginning of the next growing season.

It has been shown that coniferous seedlings grow better in the greenhouse environment with a CO_2-supplemented atmosphere than in the open (Sirén 1967, Funsch et al. 1970, Yeatmen 1970, Aldén 1971) but in this context several other aspects of these new growing methods need to be enlighted. For example:

1. How does the seedling adapt to the environmental conditions in the greenhouse?
2. How does the seedling react when the environmental conditions are abruptly changed by removing the plastic cover from the greenhouse in the late summer?
3. Are there any remaining effects, positive or negative, by growing coniferous seedlings in greenhouses instead of in the open?

In order to gather some information about these questions an extensive research program, concerning several physiological aspects of nursery grown seedlings of Scots pine and Norway spruce, is carried out at the Department of Reforestation, Royal College of Forestry, Stockholm, Sweden (cf. Aldén & Eliasson 1970, Sirén et al. 1970, Aldén 1971, Linder 1971).

The aim of the present study was to compare the physiological state of seedlings grown in the greenhouse with seedlings grown in the open. Since the pigmentation of leaves perhaps is the most widely used index of plant condition (cf. Osipova 1965) the chlorophyll and carotenoid content in needles was chosen for this comparison.

The only available information about chlorophyll content in greenhouse grown seedlings of pine and spruce is presented by Bourdeau (1959), who studied the seasonal variation in photosynthetic efficiency of coniferous seedlings grown outdoors and indoors. He found that the greenhouse grown plants had a higher content of chlorophyll during the whole investigated period.

Unusual coloration of leaves is often considered as a symptom of nutrient deficiency and can in many cases be used to identify deficiency diseases in nursery stocks (Wilde & Voigt 1952, Benzian 1965).

However, in northern provenances of Scots pine there is a pronounced seasonal variation of needle pigments and these changes can in many cases resemble changes caused by nutrient deficiencies (cf. Gerold 1959 a).

Thus, it was of special interest both to compare the pigmentation of needles from plants grown under different conditions and to follow the seasonal variation of chlorophyll and carotenoids in these seedlings.

2 Review of Literature

2.1 Seasonal Variation of Pigments in Needles of Scots Pine

The seasonal variation of color in Scots pine needles has been observed by foresters and biologists for a long time. Engler (1913) was the first one to report about the discoloration of needles during the winter. Since then many papers concerning chloroplast pigments in conifers have been published.

The fact that the needle color in the winter is related to the seed origin was shown in trials where Scots pine seedlings, from different provenances, were grown under the same environmental conditions (Langlet 1936, 1942, Baldwin 1955a, 1955b, Wright et al. 1966). It was found that plants from northerly provenances turn yellower in the winter than those of southerly provenances.

Mergen (1953) reported a significant correlation between the total chlorophyll content and nitrogen content in the needles of Scots pine and Norway spruce. On the other hand, Gerold (1959a) has shown that there was no relationship between winter foliage color and the amount of nine tested nutrient salts. By investigating the relation between microclimatic factors and seasonal discoloration (Gerold 1959b) it was found that reduction in light intensity and protection against freezing temperatures reduced the yellowing.

The influence of altitude of seed source on foliage color in Scots pine has been investigated by Baldwin (1955b) and Garret (1969). Baldwin found that high altitude sources tend to exhibit yellow-green and lowland origins blue-green needles. Garret, on the other hand, reported that the darkest foliage was found on seedlings from sources on the highest altitudes, and the yellowest foliage was found on seedlings from sources on altitudes under 300 meters.

During the past two decades Scots pine has become America's most important Christmas tree and many reports about undesirable yellow coloration of the needles in the winter have been presented (See Gerold 1959b, King 1965, White & Wright 1967).

Most of the papers referred to so far deal mainly with color changes of needles in autumn and winter. Only a few provide additional information on color changes during spring and summer.

More detailed investigations of the seasonal variation of chlorophyll throughout the year in Scots pine needles have been performed by Stålfelt (1927), Zacharowa (1929), Pravdin (1964) and Ollykainen (1967, 1969a, 1969b). Stålfelt (1927) reported and Zacharowa (1929) supported his conclusions that chlorophyll content increased to a maximum in December or January, then dropped sharply until March, reached another maximum in June and decreased again until August. On the other hand, Pravdin (1964) and Ollykainen (1967, 1969a, 1969b) reported a maximum of chlorophyll content at the end of the growing season and a minimum during late winter. In one-year-old needles of Scots pine, Laurs (1935) found that the chlorophyll concentration increased continually from July to March when he followed the pigment variations throughout a mild winter in Aachen, Germany. Berner (1949) did not notice any pronounced seasonal variation of chlorophyll in nursery grown seedlings of Scots pine and Norway spruce.

Langlet (1942) investigated the seasonal variation of carotenoids in pine needles and found that the carotenoid content increased gradually from July to January. Kaloudin

(1967) reported a higher concentration of carotenoids in April than June. Ollykainen (1967, 1969a), on the other hand, found that the carotenoid content increased during the vegetation period until cessation of growth and then decreased during the winter.

Wettstein-Westerheim and Grüll (1954) have shown that winter coloration in pine seedlings can be induced by short-day treatment.

2.2 Seasonal Variation of Pigments in Needles of Norway Spruce

Data concerning the relationship between origin of seed source and content of chlorophyll in needles of Norway spruce are, as far as the author is informed, not available.

Some rather contradictionary reports on the seasonal variation of pigments in needles of Norway spruce have been published. Stålfelt (1927) and Zachaowa (1929) reported that spruce needles had a maximum of chlorophyll in winter and summer and minima in spring and autumn. Atanasiu (1968), who followed the variation of chlorophyll in some conifers from October until April, obtained the highest concentration of chlorophyll at the end of November. For Norway spruce, as for Scots pine, Ollykainen (1969b, 1970) found the highest concentration of both green and yellow pigments at the end of the growing season. No decline in the content of chlorophyll in the needles of spruce during the winter was found by Laurs (1935), Zeller (1936) and Godnev et al. (1969).

2.3 Other Aspects on Pigmentation in Needles of Scots Pine and Norway Spruce

Investigations of chloroplast pigments in Scots pine and Norway spruce concerning other aspects than variations due to provenance and time of the year have been performed. Some of these papers are of interest for the present study and are therefore mentioned.

Wieckowski and Goodwin (1966, 1967) studied the metabolism of chlorophyll and carotenoids in cotyledons of four species of pine, including Scots pine, grown in light and in darkness. They found that the ratio chlorophyll:carotenoids was lower in darkness than in light, which means that relatively more carotenoids are synthesized in the absence of light. The influence of low temperature on the biosynthesis of chlorophyll in needles of Norway spruce was studied by Godnev and Hodasevic (1965). They reported that no synthesis of pigments took place below —2 °C.

The effect of shading upon the content of chlorophyll in seedlings of Scots pine and Norway spruce was investigated by Berner (1949). He found that when reducing the light level the chlorophyll content increased. The largest increase of chlorophyll was found at the lowest light level (20 % of incoming light) and the increase was larger in spruce than in pine.

The influence of tree age and needle age on the content of α- and β-carotene in needles of nine coniferous species, including Scots pine and Norway spruce, was studied by Kaloudin and Kaloudin (1967). They found that needles from young trees (20 years old) had a higher carotene content than needles from old trees (80 years old). One-year-old needles on all studied trees had a lower content of carotene than two-year-old needles.

3 Material and Methods

3.1 **Plant Material**

The older plant material consisted of potted seedlings of Scots pine (*Pinus silvestris* L.) and Norway spruce (*Picea abies* (L.) Karst.) which at the beginning of the investigation were one and two years old (Table 1). Germinants sown in a seedbed of Sphagnum peat were studied as soon as primary needles appeared.

In spite of the increasing age of both plants and needles during the investigation they are called current-, one- and two-year-old in respect to their age in June 1969. Both primary and secondary leaves are called needles in this report (Cf. Mirov 1967).

The seeds for the plant material were collected in natual stands close to the places mentioned in Table 1.

The plants were grown in a research nursery in northern Sweden, latitude 65°48′ N., longitude 20°34′ E. at an altitude of 80 meters. The seedlings were cultivated according to the standard routine for the nursery. During the summer months May through August plants were grown inside plastic greenhouses under controlled CO_2, water and nutrition conditions. Peat, mixed with 1 kg dolomite/m^3 peat, was used as growth substrate and nutrients were supplied in solution through the irrigation system (Wallco L-65/13; for the composition see Ingestad 1967).

In the middle of August the plastic cover was removed from the greenhouses and the seedlings were then exposed to outdoor conditions until the next spring.

Early in the spring of 1969, before covering the greenhouses with plastic, 500 seedlings from each age class (1 and 2 years) were moved to a place outside the greenhouses. The plants were distributed as 100 seedlings/m^2. On the same day one provenance of Scots pine and one provenance of Norway spruce were sown both in the greenhouse and outdoors. Another two provenances of Scots pine were sown only in the greenhouse (Table 1).

Plants inside and outside the plastic greenhouse were treated in the same manner with respect to water, nutrition, fungicides, insecticides etc.

3.2 **Collection of Needle Samples**

Ten randomly chosen seedlings (more fore current year seedlings were collected at approximately ten days' interval during the summers of 1969 and 1970. During the winter 69/70 after the seedlings were covered with snow samples were taken less often (cf. Table 2).

To reduce the effect of daily fluctuations in the content of pigments (see Mitrakos 1959, Sironval 1963) and variations in fresh weight (Langlet 1936) samples from all plots were collected at the same time of the day and stored in darkness at +4° C until the pigments were extracted (Sestak 1959).

From the terminal leaders of the ten seedlings all needles of the same age were removed, cut into small pieces and carefully mixed. It was assumed that the mixed sample would reduce the effect of variation in pigment content along the needles (Wood & Bachelard 1969) and the individual variability of the seedlings.

3.3 **Extraction of Pigments**

For extraction 150 mg of needles, fresh weight, were used. This amount of needles gave a pigment concentration in the solution which was in the region of absorbencies from 0.2--0.8 where the Lambert-Beer law

Table 1. Origin of seed source and years for sowing of the plant material used in the investigation.

Origin District	Latitude North	Longitude East	Altitude Meters	Sown Year
Scots pine				
Roknäs	65°21′	21°12′	0—100	1967
Gällivare	67°06′	20°40′	300—400	1967
Gällivare	67°06′	20°40′	300—400	1968
Gällivare	67°06′	20°40′	300—400	1969
Vittangi	67°35′	21°45′	300—400	1969
Korpilombolo	66°52′	23°02′	300—400	1969
Norway spruce				
Skellefteå	64°46′	21°00′	200—300	1967
Lycksele	64°32′	18°50′	300—400	1967
Nederkalix	65°51′	23°09′	0—100	1968
Nederkalix	65°51′	23°09′	0—100	1969

Table 2. Days when seedlings used in pigment analysis were collected. Spruce seedlings were always collected on the first of the two days.

1969						1970							
June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Febr.	Mar.	Apr.	May	June	July
6	3	3	19	26		6		21		4	11	2	7
7	4	4	20	27		7		22		5	12	3	8
16	14	12									17	9	14
17	15	13									18	10	15
26	22	26									27	16	
27	23	27									28	17	
												28	
												29	

is obeyed in chlorophyll determinations (Cf. Bruinsma 1963).

The needle pieces were ground in 100 % acetone (with some $MgCO_3$ present to buffer the solution) in a mortar. The extract and the macerated tissue were then filtered through a glass filter and then made up to exactly 50 ml (see Hoffman & Werner 1966, Wintermans 1969). To avoid destruction of the pigments by photo-oxidation or chlorophyllase activity extractions were done in dim dark-room light (Filter: Gevaert L 501) and the mortar was kept in ice during the extraction (see Mackinney 1940, Bruinsma 1963). The pigment extracts were stored in darkness at +4° C until measured in a Shimadzo Multipurpose Recording Spectrophotometer model MPS-50L.

The concentrations of chlorophylls a and b and total carotenoids were determined from the formulae given by Holm (1954).

$$C_a = 9.78 \cdot A_{662} - 0.99 \cdot A_{644}$$

$$C_b = 21.4 \cdot A_{644} - 4.65 \cdot A_{662}$$

$$C_{car} = 4.69 \cdot A_{440.5} - C_{(a+b)} \cdot 0.267$$

A = Absorbence and C = mg pigment per liter solution.

The grouping together of all yellow pigments into one class is a source of some dissatisfaction, but their absorption coefficients are unfortunately too similar to permit their separation in a mixed solution by spectrophotometric methods.

All values were calculated as mg pigment per g fresh or dry weight. For the statistical treatment of the results the Wilcoxon signed rank test was used. (Cf. Snedecor & Cochran 1967).

3.4 **Meteorological Data**

The climate inside and outside the greenhouses was recorded at short intervals with the help of a data-logging system (Backlund & Perttu 1971). The distribution of quanta within the visible part of the spectrum was measured with a Quantaspectrometer QSM 2400 (Incentive Research and Development AB, Bromma, Sweden) connected to a Goerz Electro RE 511 Recorder.

4 Results

All seedlings investigated showed a similar pattern in respect to seasonal variation, response to greenhouse climate and relation between needles of different ages. Only one provenance of Scots pine (Roknäs) and one for Norway spruce (Skellefteå) are therefore chosen to illustrate the variations graphically, but values for all seedlings together are presented in tables.

In spite of the individual variability of pigment concentration between seedlings, there was a good agreement between the average value for ten seedlings and the value obtained with one sample from a needle mixture (Table 3).

4.1 Seasonal Variation of Pigments in Needles from Two-Year-Old Seedlings of Scots Pine

4.1.1 *Chlorophyll*

Roknäs: Current and one-year-old needles from greenhouse-grown seedlings had a pronounced increase of chlorophyll during the summer with maxima in the beginning of August (Figure 1). From the last week in August there was a steep decrease until the middle of October. The plastic cover of the greenhouse was removed on August 16 and the plants were covered with snow from the last week in September 1969 until the first week in May 1970. During this period a slight increase in chlorophyll content was found. In spring, after the snow had melted, the chlorophyll concentration was decreasing until the greenhouse was covered with plastic on May 27, 1970. Thereafter the chlorophyll content increased sharply.

During the summer of 1969 current year needles had a higher content of chlorophyll than older needles when expressed on a dry weight basis. At the end of August when the current year needles were mature, there was an inverse relationship and throughout the rest of the period the older needles had the higher content of chlorophyll. Older needles had a higher content of chlorophyll during the whole investigated period when the content of pigment was calculated on a fresh weight basis (Figure 2 and Table 4). The difference between Figures 1 and 2 therefore can be explained by the lower ratio dry weight : fresh weight for the immature needles.

When comparing the chlorophyll content in current year needles from plants inside or outside the greenhouse (Figure 3), greenhouse-grown seedlings had a much higher concentration of chlorophyll than seedlings grown in the open when calculated on a dry weight basis, sometimes more than 100 %. The difference remained even after the plastic cover was removed from the greenhouse. If the pigment content was expressed on a fresh weight basis the difference still existed for current year needles but was not significant for one-year-old needles (Table 4), due to the higher ratio dry weight : fresh weight for needles from seedlings grown in the open. The relation between the chlorophyll concentrations in current and one-year-old needles from seedlings grown in the open showed the same relationship as for greenhouse-grown plants (Figures 1—2).

Gällivare: This provenance showed similar variations and differences to those presented for Roknäs (Figures 1—3). Current year needles had the highest content of chlorophyll during the summer of 1969 when calculated on a dry weight basis and older needles had a higher content during the whole period when calculated instead on a fresh weight basis (Table 4). Greenhouse-grown seedlings had a much higher content of chlorophyll than seedlings grown in the

Table 3. Results from a comparison between the average value obtained from ten individually analysed seedlings and the 'average value' obtained by analysing one mixed sample.

Seedling No.	mg chl / g dw	mg car / g fw	mg chl / g dw	mg car / g fw	chl / car	dw / fw
1	2.62	1.02	1.26	0.49	2.47	0.48
2	2.66	0.94	1.26	0.44	2.83	0.47
3	2.29	0.91	1.11	0.44	2.52	0.49
4	2.51	1.01	1.21	0.48	2.49	0.48
5	2.06	0.85	0.99	0.41	2.44	0.48
6	2.86	1.05	1.33	0.49	2.72	0.47
7	2.03	0.83	0.95	0.39	2.45	0.47
8	2.28	0.89	1.05	0.41	2.56	0.46
9	1.65	0.72	0.77	0.34	2.29	0.47
10	2.38	0.88	1.18	0.43	2.71	0.49
Average	2.33	0.91	1.11	0.43	2.55	0.48
Standard error	4.82 %	3.47 %	4.90 %	3.42 %	2.00 %	0.63 %
Mixed sample 1	2.32	0.91	1.11	0.43	2.56	0.48
Mixed sample 2	2.33	0.91	1.12	0.44	2.55	0.48

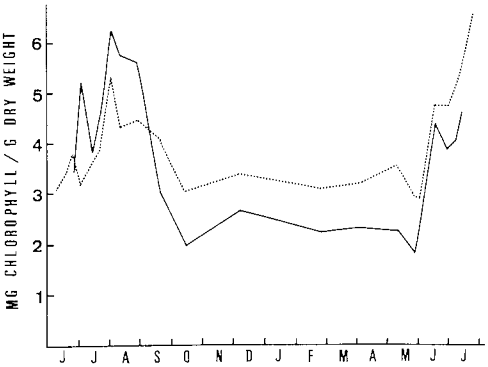

Figure 1 Seasonal variation of chlorophyll in needles from 'greenhouse' grown seedlings of Scots pine, from June 1969 to July 1970. Chlorophyll content in mg chlorophyll per g dry weight. —— current year needles, ---- one-year-old needles.

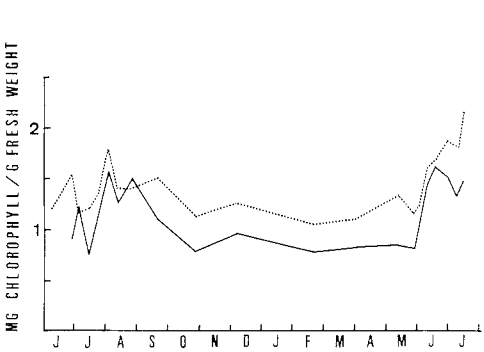

Figure 2 Seasonal variation of chlorophyll in needles from 'greenhouse' grown seedlings of Scots pine, from June 1969 to July 1970. Chlorophyll content in mg chlorophyll per g fresh weight. —— current year needles, ---- one-year-old needles.

open for both current and one-year-old needles when expressed on a dry weight basis (Table 4). Calculated on a fresh weight basis the content of chlorophyll was significantly higher for current year needles from greenhouse-grown plants but not for the older needles due to the higher ratio dry weight : fresh weight for needles from plants grown in the open (Table 4).

When comparing the two provenances Roknäs and Gällivare it was found that current year needles from greenhouse-grown seedlings from Roknäs had a higher chlorophyll content than those from the Gällivare plants when calculated as mg pigment per g dry weight (Table 5). There was no significant difference if calculated instead on a fresh weight basis. For current year needles from plants grown in the open there was no significant difference in chlorophyll content between the two provenances.

Older needles both from the greenhouse

Table 4. Results from significance test of differences in pigmentation and ratio dry weight : fresh weight between seedlings of Scots pine grown under different nursery conditions. Level of significance: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***). The position of the stars indicates the group with the higher content or ratio.

Growing conditions		Greenhouse						Outdoors					
	Needle age	Current year			One year			Current year			One year		
Origin	Plant age	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw
Roknäs	2 years	***	***		***					**			***
Gällivare	2 years	***	***		**					*			**
Gällivare	1 year	***	***		***								**
Gällivare	current	*	**										
		mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car
Roknäs	2 years	***	***	***	**		***						
Gällivare	2 years	***	***	**	***	**							
Gällivare	1 year	***	***	***	***	*	**						
Gällivare	current			**									

Table 5. Results from significance test of differences in pigmentation and ratio dry weight: fresh weight between two provenances of Scots pine seedlings grown under different nursery conditions. Level of significance: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***). The position of the stars indicates the group with the higher content or ratio.

Provenance	Roknäs						Gällivare					
Needle age	Current year			One year			Current year			One year		
Growing place	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw
Greenhouse	*			***	***							*
Outdoors				**	***							
	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car
Greenhouse	**			***	***							
Outdoors				***	***							*

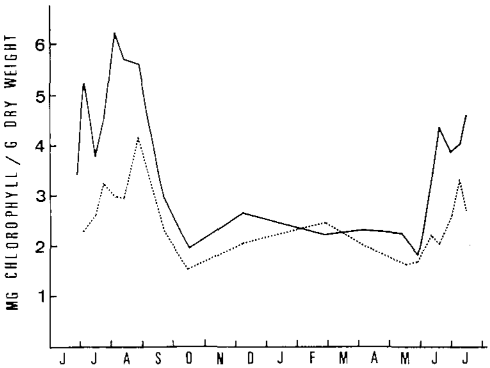

Figure 3 Seasonal variation of chlorophyll in current year needles from Scots pine seedlings grown under different nursery conditions. Chlorophyll content in mg chlorophyll per g dry weight. —— 'greenhouse' grown seedlings, - - - - seedlings grown in the open.

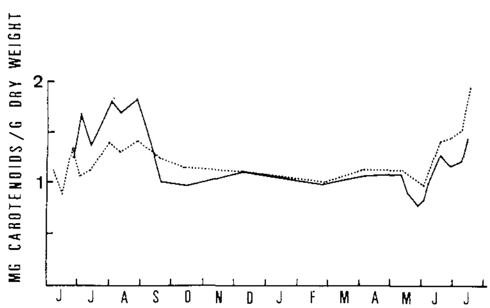

Figure 4 Seasonal variation of carotenoids in needles from 'greenhouse' grown seedlings of Scots pine, from June 1969 to July 1970. Carotenoid content in mg carotenoids per g dry weight. —— current year needles, - - - - one-year-old needles.

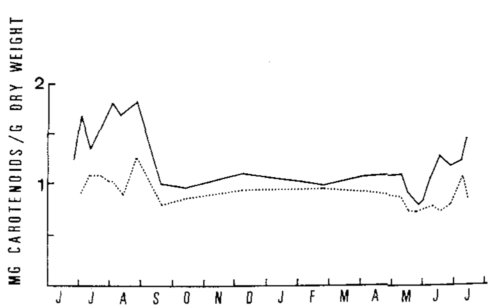

Figure 5 Seasonal variation of carotenoids in current year needles from Scots pine seedlings grown under different nursery conditions. Carotenoid content in mg carotenoids per g dry weight. —— 'greenhouse' grown seedlings, - - - - seedlings grown in the open.

and outdoors had significantly higher chlorophyll concentration for Roknäs (Table 5). This was valid for both dry and fresh weight values.

4.1.2 *Carotenoids*

Roknäs: The carotenoid concentration in greenhouse-grown seedlings increased during summer with a maximum concentration of carotenoids for both ages of needles at the end of August (Figure 4). From September to December a slight increase was recorded, but the carotenoid concentration was rather constant during the winter when the seedlings were covered with snow. In May, when the snow disappeared, the carotenoid level for both current and one-year-old needles decreased until the greenhouse was covered with plastic again.

As for chlorophyll (Figure 1) the current year needles had a higher content of carotenoids during the first summer when calculated on a dry weight basis. In the autumn, however, when the current year needles were mature, the one-year-old needles showed the highest concentration of carotenoids both on dry and fresh weight basis.

Both current and one-year-old needles had a higher concentration in needles from seedlings grown in the greenhouse than those grown in the open (Figure 5 and Table 4). When calculated on a fresh weight basis the difference was not significant for one-year-old needles during the whole period (Table 4). As already mentioned, the needles from outdoors had a higher ratio dry weight : fresh weight than needles from the greenhouse-grown seedlings.

Gällivare: The difference in carotenoid content between plants grown in the open and plants grown in the greenhouse was similar to those already described for Roknäs (Figure 5 and Table 4). The seasonal variations showed a similar pattern as well (Cf. Figures 4 and 5). The comparison of the two provenances, Roknäs and Gällivare, showed that one-year-old needles from Roknäs had a significantly higher concentration of carotenoids both inside and outside the green-

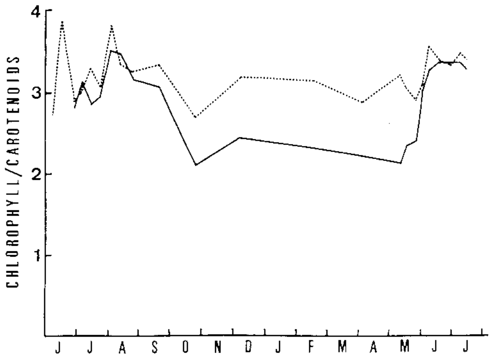

Figure 6 Seasonal variation of the ratio chlorophyll : carotenoids in needles from 'greenhouse' grown seedlings of Scots pine, from June to July 1970. —— current year needles, - - - - one-year-old needles.

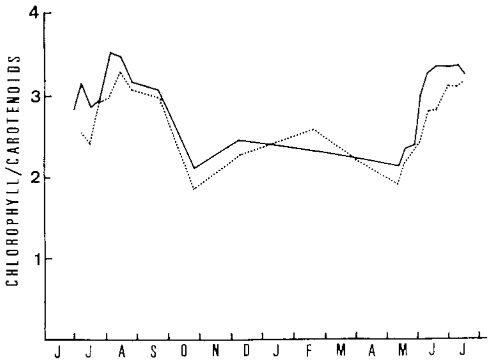

Figure 7 Seasonal variation of the ratio chlorophyll : carotenoids in current year needles from Scots pine seedlings grown under different nursery conditions. —— 'greenhouse' grown seedlings, - - - - seedlings grown in the open.

house (Table 5). The difference between current year needles was not that clear and only needles from the greenhouse-grown seedlings had a significantly higher level of carotenoids for Roknäs when expressed as mg pigment per g dry weight.

4.1.3 *Ratio chlorophyll:carotenoids*

The seasonal variation of the ratio chlorophyll:carotenoids for current and one-year-old needles from seedlings grown in the greenhouse had a maximum in summer and a minimum during the winter (Figure 6). The older needles had a higher ratio throughout the whole period. Greenhouse-grown seedlings had a significantly higher ratio chlorophyll:carotenoids than seedlings grown in the open (Figure 7 and Table 4). The difference between the two provenances was not so pronounced, but one-year-old needles from Gällivare seedlings grown in the open had a little higher ratio than seedlings from Roknäs.

4.1.4 *Seasonal variation of pigments in needles from current and one-year-old seedlings of Scots pine*

All seedlings included in the investigation had a similar pattern of seasonal variations of pigments and also a similar relationship in pigmentation of needles of different age. These variations have been described in Figures 1—8 and in Tables 4 and 5 and therefore only the influence of growing place and plant age will be considered for the remaining groups.

There were no significant differences in either chlorophyll or carotenoid content between the three tested provenances of greenhouse-grown current year seedlings. Therefore only one provenance, Gällivare, will be used for the further comparisons.

All seedlings independent of plant age had a higher content of chlorophyll during the whole investigated period when exposed to greenhouse climate for ten weeks a year (Table 4). On the other hand, needles from these plants had a lower ratio dry weight: fresh weight than needles from plants grown in the open (Table 4). The concentration of yellow pigments was also significantly higher for greenhouse-grown seedlings except for current year seedlings where no significant difference was obtained (Table 4). The ratio chlorophyll:carotenoids was higher in needles from plants grown in the greenhouse.

4.1.5 *Influence of plant age upon the pigmentation in needles of Scots pine*

Seedlings from the same provenance, Gällivare, but with different age, current-, one and two years old, were used to determine the effect of plant age upon the pigmentation of needles (Tables 6—8).

Table 6. Results from significance test of differences in pigmentation and ratio dry weight : fresh weight between one- and two-year-old seedlings of Scots pine grown under different nursery conditions. Level of significance: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***). The position of the stars indicates the group with the higher content or ratio.

Origin, plant age	Gällivare 2 years						Gällivare 1 year					
Needle age	Current year			One year			Current year			One year		
Growing place	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw
Greenhouse							**	*				
Outdoors									*			*
	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car
Greenhouse							**	**	*	**	***	
Outdoors							***			**	***	

Table 7. Results from significance test of differences in pigmentation and ratio dry weight : fresh weight between current and two-year-old seedlings of Scots pine grown under different nursery conditions. Level of significance: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***). The position of the stars indicates the group with the higher content or ratio.

Origin, plant age	Gällivare 2 years			Gällivare current year		
Needle age	current year			current year		
Growing place	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw
Greenhouse			***	**		
Outdoors			***	**		
	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car
Greenhouse				***	**	
Outdoors				***	**	

Table 8. Results from significance test of differences in pigmentation and ratio dry weight : fresh weight between current and one-year-old seedlings of Scots pine grown under different nursery conditions. Level of significance: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***). The position of the stars indicates the group with the higher content or ratio.

Origin, plant age	Gällivare 1 year			Gällivare 2 years		
Needle age	Current year			Current year		
Growing place	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw
Greenhouse			***	*		
Outdoors			***	***	**	
	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car
Greenhouse			*	***		
Outdoors				***	***	

Current year needles from one-year-old, greenhouse-grown, seedlings had significantly higher content of both chlorophyll and carotenoids than needles of the same age from two-year-old seedlings (Table 6). For seedlings grown in the open there was no significant difference in pigmentation of the current year needles. One-year-old needles from one-year-old seedlings had significantly higher content of carotenoids than needles from the older plants both when grown inside or outside the greenhouse. There was no difference in the content of chlorophyll due to plant age for the one-year-old needles.

Current year needles from current year seedlings had a higher content of both chlorophyll and carotenoids than current year needles from two-year-old plants (Table 7) and one-year-old plants (Table 8). Noticing

Table 9. The content of chlorophyll in current and one-year-old needles from Scots pine seedlings grown in greenhouse with normal and enriched CO_2-atmosphere.

Greenhouse atmosphere		Enriched CO_2		Normal CO_2	
	Sampling date	July, 10	July, 25	July, 10	July, 25
Plant age	Needle age	mg chl / g dw	mg chl / g dw	mg chl / g dw	mg chl / g dw
1 year	current	4.51	4.52	4.77	4.51
1 year	one year	6.16	5.39	5.39	5.66
2 years	current	4.00	3.59	4.39	4.86
2 years	one year	5.15	4.90	4.78	5.38

Table 10. The content of chlorophyll in current and one-year-old needles from Scots pine seedlings grown in greenhouse with normal and double dose of nutrients.

Dose of nutrients		Normal		Double	
	Sampling date	July, 10	July, 25	July, 10	July, 25
Plant age	Needle age	mg chl / g dw	mg chl / g dw	mg chl / g dw	mg chl / g dw
1 year	current	4.77	4.51	4.40	5.64
1 year	one year	5.39	5.66	6.36	7.73
2 years	current	4.39	4.86	4.06	4.41
2 years	one year	4.78	5.38	4.65	5.52

that one-year-old needles from one-year-old seedlings and current year needles from current year seedlings are of primary type (juvenile) while the other needles are of secondary type (adult), the differences obtained might be due to other reasons than plant age.

4.1.6 *Effect of CO_2-supplemented atmosphere and influence of fertilizers on the chlorophyll content in Scots pine needles*

In a preliminary survey during the summer of 1968 the effect of CO_2-supplemented atmosphere on the chlorophyll content in pine needles was studied. No particular influence of the enriched CO_2 level in the air upon the chlorophyll content in needles was found (Table 9).

It was also investigated whether the fertilization program gave an optimal concentration of chlorophyll. On each occasion when the seedlings were fertilized, half of the seedlings (500 seedlings) received a double dose of nutrients. No significant difference in chlorophyll content due to the difference in nutrient supply was obtained (Table 10).

4.2 **Seasonal Variation of Pigments in Needles from Two-Year-Old Seedlings of Norway Spruce**

4.2.1 *Chlorophyll*

Skellefteå: Both current and one-year-old needles from greenhouse-grown seedlings had a sharp increase of the chlorophyll concentration during July and August, with maximum for one-year-old needles in the middle and for current year needles at the end of August (Figure 8). Minima were found in December and May. A slight increase in the chlorophyll content was recorded during the winter.

The younger needles had a higher chlorophyll concentration, when calculated on a dry weight basis, than older ones except for a few weeks in May 1970. When instead expressed as mg chlorophyll per g fresh weight older needles had a higher chlorophyll content on most occasions (Figure 9) but the difference was not as pronounced as earlier shown for Scots pine (Figure 2).

Current year needles from greenhouse-grown seedlings had a higher content of chlorophyll during the whole investigated period than needles from seedlings grown in the open, whether calculated on a dry or fresh weight basis (Figures 10 and 11). The situation was similar for one-year-old needles (Table 11). Current year needles from the greenhouse had a higher ratio dry weight:fresh weight than needles from plants grown in the open (Table 11). For one-year-old needles there was an inverse relationship (Table 11).

Lycksele: This provenance showed a similar pattern for the seasonal variation of chlorophyll to that already shown for Skellefteå (Figures 8, 9, 10 and 11). Needles from greenhouse-grown seedlings had a higher content of chlorophyll than needles from plants grown in the open (Table 11).

When comparing the two provenances, Skellefteå and Lycksele, it was found that only for one-year-old needles from the greenhouse was there a significant difference in chlorophyll content between the provenances (Table 12). The ratio dry weight:fresh weight was significantly higher for one-year-needles from Skellefteå than Lycksele, both inside and outside the greenhouse (Table 12).

4.2.2 *Carotenoids*

Skellefteå: The content of carotenoids in current and one-year-old needles from greenhouse-grown seedlings increased during the summer with a maximum for both ages of needles in August (Figure 12). A minimum was recorded in December and after that the concentration of carotenoids remained rather constant during the winter. In May, after the snow had disappeared, the content of carotenoids decreased until the end of the month.

Greenhouse-grown seedlings had a higher content of carotenoids during the whole investigated period (Figure 13 and Table 11).

Lycksele: The differences between plants grown in the greenhouse and in the open were similar to those already described for Skellefteå (Figure 13 and Table 11). The relationship between needles of different ages was also similar.

When the two provenances were compared, both current and one-year-old needles from plants grown in the greenhouse, a higher content of carotenoids was found for Skellefteå plants than for Lycksele (Table 12). From the seedlings grown in the open only current year needles showed this difference in carotenoid content.

4.2.3 *Ratio chlorophyll:carotenoids*

Skellefteå: The ratio chlorophyll:carotenoids in current and one-year-old needles from greenhouse-grown seedlings had a maximum in the beginning of August, a decrease in the autumn and a slight increase during the winter (Figure 14). In May the ratio decreased during a few weeks before the accumulation of pigments started again. The older needles had a higher ratio than the younger ones during the whole year (Figure 14).

With a few exceptions needles from the greenhouse-grown seedlings had the highest ratio chlorophyll:carotenoids during the whole investigated period (Figure 15 and Table 11).

Lycksele: This provenance showed a similar seasonal pattern and the same relations between needles of different ages and different growing places as already shown for Skellefteå (Table 11).

Of the two provenances, Lycksele had a significantly higher ratio chlorophyll:carotenoids than Skellefteå. This difference was independent of growing place and needle age.

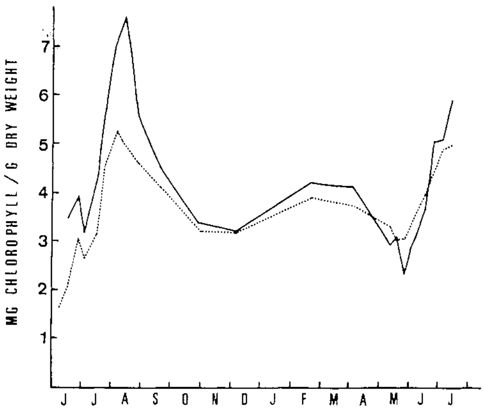

Figure 8 Seasonal variation of chlorophyll in needles from 'greenhouse' grown seedlings of Norway spruce, from June 1969 to July 1970. Chlorophyll content in mg chlorophyll per g dry weight. —— current year needles, ---- one-year-old needles.

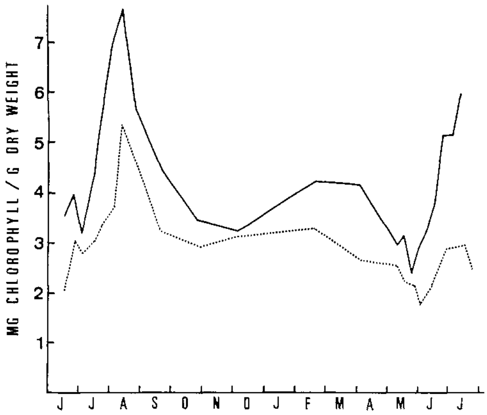

Figure 10 Seasonal variation of chlorophyll in current year needles from seedlings of Norway spruce grown under different nursery conditions. Chlorophyll content in mg chlorophyll per g dry weight. —— 'greenhouse' grown seedlings, ---- seedlings grown in the open.

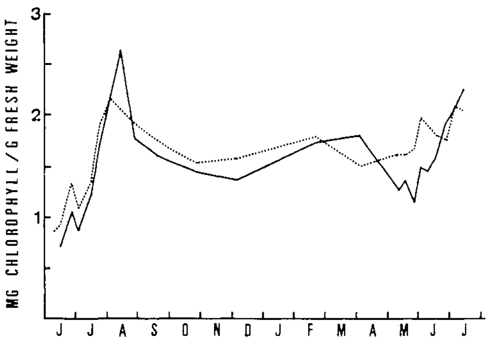

Figure 9 Seasonal variation of chlorophyll in needles from 'greenhouse' grown seedlings of Norway spruce, from June 1969 to July 1970. Chlorophyll content in mg chlorophyll per g fresh weight. —— current year needles, ---- one-year-old needles.

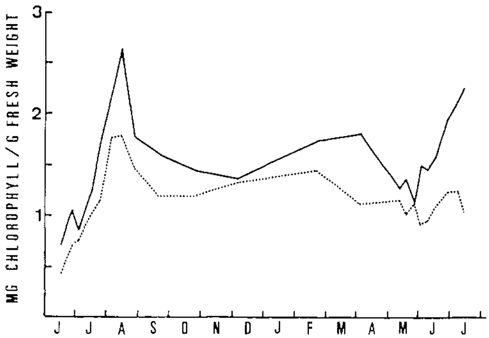

Figure 11 Seasonal variation of chlorophyll in current year needles from seedlings of Norway spruce grown under different nursery conditions. Chlorophyll content in mg chlorophyll per g fresh weight. —— 'greenhouse' grown seedlings, ---- seedlings grown in the open.

4.2.4 *Seasonal variation of pigments in needles from current and one-year-old seedlings of Norway spruce*

The studied provenance for current and one-year-old seedlings of Norway spruce was Nederkalix (Cf. Table 1).

The greenhouse-grown seedlings always had the higher content of chlorophyll when any significant difference between plants from the two growing places was obtained (Table 11). One-year-old seedlings had significantly higher contents of carotenoids when grown in the greenhouse (Table 11). On the contrary, no significant difference in the content of carotenoids between plants grown inside or outside the greenhouse was found for current year seedlings.

Greenhouse-grown seedlings had a higher ratio chlorophyll:carotenoids for both current and one-year-old seedlings (Table 11).

Table 11. Results from significance test of difference in pigmentation and ratio dry weight : fresh weight between seedlings of Norway spruce grown under different nursery conditions. Level of significance: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***). The position of the stars indicates the group with the higher content or ratio.

	Growing place	Greenhouse						Outdoors					
	Needle age	Current year			One year			Current year			One year		
Origin	Plant age	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw
Skellefteå	2 years	***	***	***	***	***							***
Lycksele	2 years	***	***	***	***	**							***
Nederkalix	1 year	***	***		***	***							*
Nederkalix	current	**											
		mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car
Skellefteå	2 years	***	***	***	***	***	***						
Lycksele	2 years	***	***	***	***		**						
Nederkalix	1 year	***	**	***	**	**	***						
Nederkalix	current			***									

Table 12. Results from significance test of difference in pigmentation and ratio dry weight : fresh weight between two provenances of Norway spruce seedlings grown under different nusery conditions. Level of significance: $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***). The position of the stars indicates the group with the higher content or ratio.

Provenance	Lycksele						Skellefteå					
Needle age	Current year			One year			Current year			One year		
Growing place	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw
Greenhouse											**	***
Outdoors												***
	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car
Greenhouse			**			*	*	***		*	***	
Outdoors			*			**	**	**				

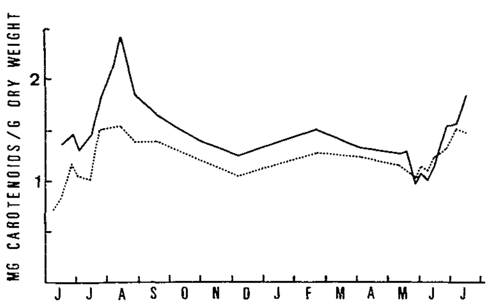

Figure 12 Seasonal variation of carotenoids in needles from 'greenhouse' grown seedlings of Norway spruce, from June 1969 to July 1970. Carotenoid content in mg carotenoids per g dry weight. —— current year needles, ---- one-year-old needles.

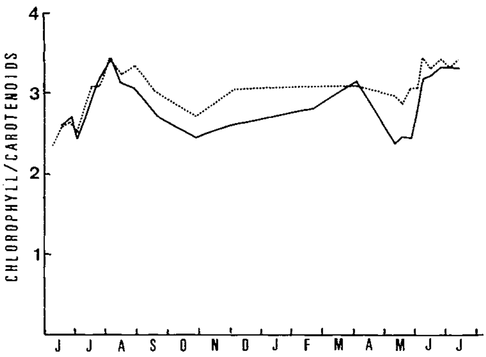

Figure 14 Seasonal variation of the ratio chlorophyll : carotenoids in needles from 'greenhouse' grown seedlings of Norway spruce, from June 1969 to July 1970. —— current year needles, ---- one-year-old needles.

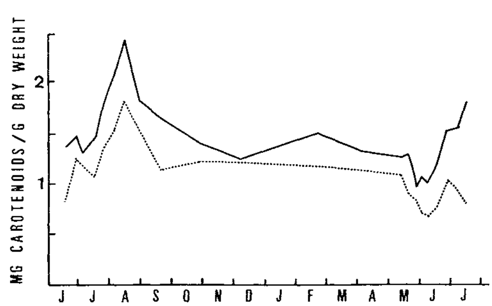

Figure 13 Seasonal variation of carotenoids in current year needles from seedlings of Norway spruce grown under different nursery conditions. Carotenoid content in mg carotenoids per g dry weight. —— 'greenhouse' grown seedlings, ---- seedlings grown in the open.

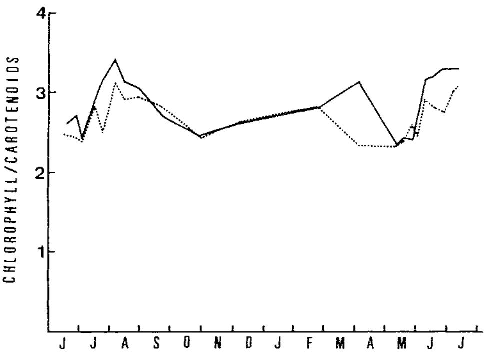

Figure 15 Seasonal variation of the ratio chlorophyll : carotenoids in current year needles from seedlings of Norway spruce grown under different nursery conditions. —— 'greenhouse' grown seedlings, ---- seedlings grown in the open.

4.2.5 *Influence of plant age upon the pigmentation in needles of Norway spruce*

Two-year-old seedlings from Nederkalix, or current and one-year-old seedlings from the other two provenances were not available at the start of the investigation and therefore it was not possible to make a similar study of the influence of plant age as for Scots pine (Cf. Table 1).

In the greenhouse there was no difference in chlorophyll content between current and one-year-old seedlings from Nederkalix (Table 13). In the open current year needles from current year seedlings had a higher content of both chlorophyll and carotenoids (Table 13). Current year needles from one-year-old seedlings had a higher ratio dry weight:fresh weight than needles from current year seedlings both when grown inside and outside the greenhouse (Table 13).

Table 13. Results from significance test of differences in pigmentation and ratio dry weight : fresh weight between current and one-year-old seedlings of Norway spruce grown under different nursery conditions. Level of significance: p < 0.05 (*), p < 0.01 (**), p < 0.001 (***). The position of the stars indicates the group with the higher content or ratio.

Origin, plant age	Nederkalix 1 year			Nederkalix current year		
Needle age	Current year			Current year		
Growing place	mg chl / g dw	mg chl / g fw	dw / fw	mg chl / g dw	mg chl / g fw	dw / fw
Greenhouse			***			
Outdoors			*	***	*	
	mg car / g dw	mg car / g fw	chl / car	mg car / g dw	mg car / g fw	chl / car
Greenhouse						
Outdoors				***	*	

5 Discussion

5.1 Seasonal Variation of Needle Pigments

A pronounced seasonal variation in the content of needle pigments in seedlings of Scots pine and Norway spruce is shown in Figures 1 to 15. Needles of both pine and spruce seedlings had a maximum of chlorophyll and carotenoids in August, a minimum during the winter and another decline of pigment concentration in spring after the snow had melted. These results confirm most of the earlier findings on chlorophyll variations in trees of Scots pine and Norway spruce (Langlet 1942, Gerold 1959 ab, Pravdin 1964, Ollykainen 1967, 1969ab, 1970). For carotenoids, Ollykainen (1967, 1969ab, 1970) has reported a decrease during the winter while other authors have reported an increase of yellow pigments during this period (Langlet 1942, Gerold 1959a, Kaloudin & Kaloudin 1967).

The content of pigments did not increase constantly during the whole growing season (Figures 1—15). In spite of a gradual increase during the summer, there were several 'ups and downs' before the maximum in August was reached. Some of these temporary declines seem to be correlated to temperature (Figures 16 and 17), but some of them are probably related to growth activities such as rapid shoot and needle development. The shoot growth took place during the first three weeks in June for most of the studied pine seedlings, both in the greenhouse and in the open. Ollykainen (1967) reported a decrease in the content of chlorophyll in needles during the period of flowering. It can therefore be assumed that even other activities could influence the content of chlorophyll in the needles.

The rapid decrease in the content of chlorophyll in the autumn is related to both day-length (Wettstein-Westerheim & Grüll 1954) and low temperature (Gerold 1959b, Pravdin 1964). Godnev and Hodasevic (1965) found that no synthesis of chlorophyll took place at temperatures below —2° C. After the plants were covered with snow at the end of September there was a decrease in both chlorophyll and carotenoid content during the first month and thereafter a slight increase during the rest of the winter months. Tranquillini (1957) also reported an increase in chlorophyll content during the winter in needles covered with snow and like White and Wright (1967) that needles on branches under snow were greener than on branches above the snow cover.

Tranquillini (1957) measured the temperature in needles under a deep snow cover and found that, in spite of air temperatures of —20 to —30° C, the needle temperature was almost constant around ±0° C. Since it is almost dark under 60 centimeters of snow (Tranquillini 1957), the conditions under the snow cover are excellent for storage of chlorophyll samples (Cf. Sestak 1959).

The respiration in needles of conifers still occurs at several degrees below zero (Polster & Fuchs 1962, Godnev & Hodasevic 1965). This is to say that there is a constant loss of stored reserve food and therefore a decrease in dry weight, while at the same time the concentration of pigments is relatively unaffected. The recorded increase of pigments in needles under the snow therefore might be a false increase. The problem is that the reference basis for the calculation of pigment content is not constant when using fresh weight or dry weight as a basis. One way to avoid this problem should be to express the pigment content on an individual needle basis. Because the length of the needles differed so much between plants grown in the greenhouse and in the open (Table 14), needles were not a suitable basis

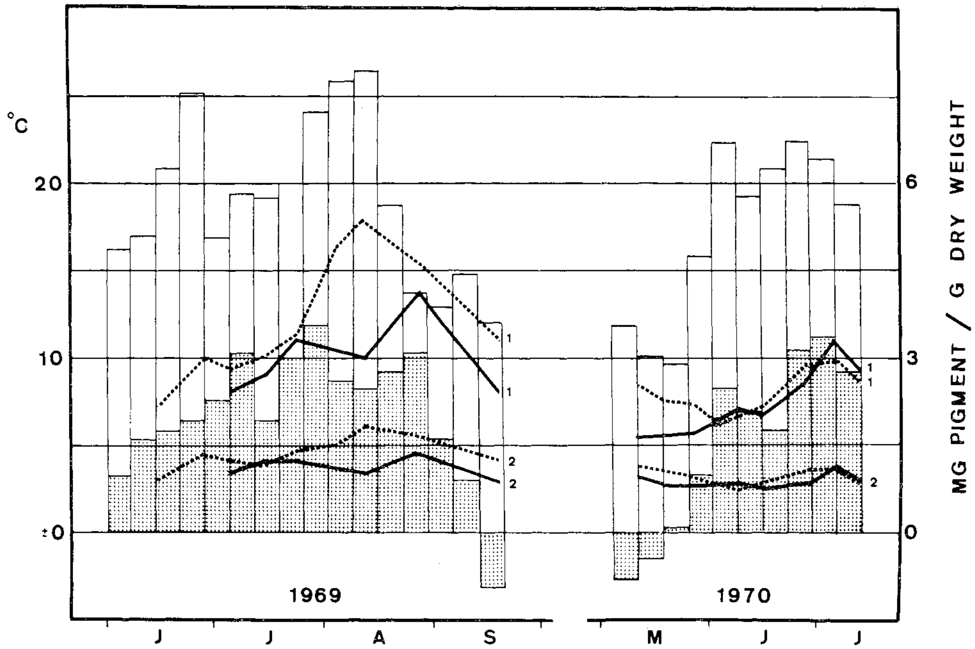

Figure 16 The weekly average minimum (dotted) and maximum (open) temperatures in the open, June—September 1969 and May—July 1970. Content of chlorophyll (1) and carotenoids (2) during the same periods in current year needles of Scots pine (——) and Norway spruce (- - - -) seedlings grown in the open.

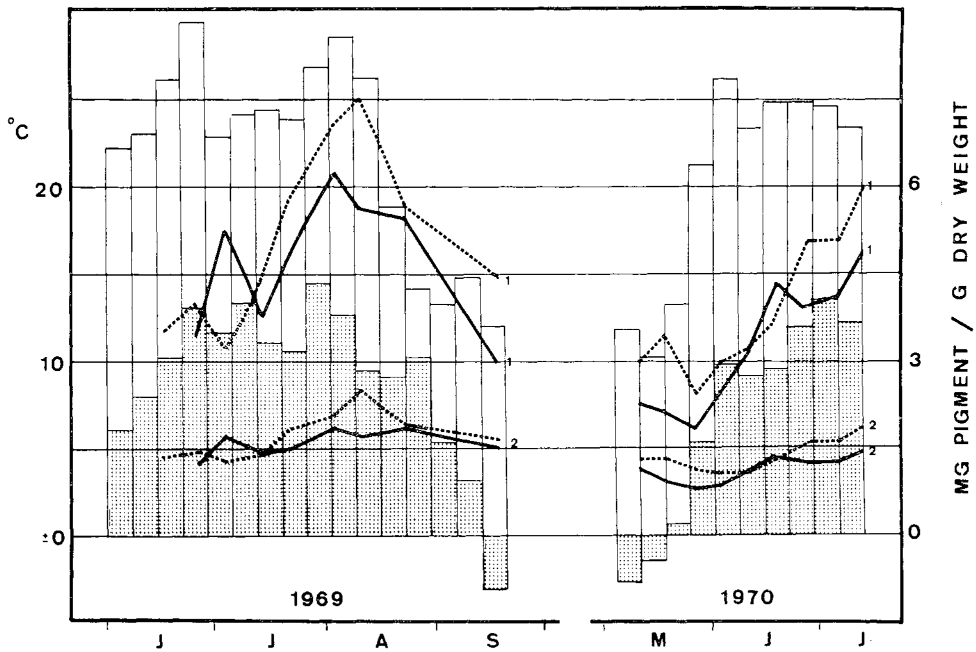

Figure 17 The weekly average minimum (dotted) and maximum (open) temperatures 'in' the greenhouse, June—September 1969 and May—July 1970. Content of chlorophyll (1) and carotenoids (2) during the same periods in current year needles of Scots pine (——) and Norway spruce (- - - -) seedlings grown in the greenhouse.

Table 14. Length of current year needles from two-year-old seedlings of Scots pine grown under different nursery conditions. Average of 150 needles.

Provenance	Gällivare				Roknäs			
Growing place	Greenhouse		Outdoors		Greenhouse		Outdoors	
Year	1969	1970	1969	1970	1969	1970	1969	1970
Length in mm	104	105	65	77	105	116	83	67
Standard error %	0.3	0.4	0.6	0.5	0.8	0.6	0.4	0.8

when comparing seedlings grown under different conditions.

The discoloration of pine needles in the autumn could be due to a decrease in the concentration of pigments (Figures 1—6), a grouping together of the chloroplasts (Parker & Philpot 1961, 1963), disruption of the chloroplasts and release of the pigments into the cytoplasm (Perry & Baldwin 1966) but it might also be an effect of the decreased ratio chlorophyll : carotenoids (Figures 6 and 7).

In spruce needles also a decrease in the ratio chlorophyll:carotenoids was recorded but was less pronounced than for pine needles (Figures 14 and 15).

The sharp decrease in the content of pigments in May, after the snow had melted, could be due to photo-oxidation of the inactive pigments which during the winter had been released from the chloroplasts into the cytoplasm and there stored under favorable conditions (Cf. Perry & Baldwin 1966).

5.2 Influence of Seed Origin upon the Content of Needle Pigments

Most of the information on differences in inherent color of Scots pine needles comes from provenance tests (Cf. Langlet 1936, 1942, Baldwin 1955ab, Gerold 1959ab, Wright et al. 1966). Engler (1913) recognized that intensity and earliness of winter discoloration in Scots pine needles are correlated with latitude and altitude of the seed source and little has been added since then.

The provenances studied in this investigation had, compared with earlier trials, only small differences in latitude. The most differing factor between the seed sources was altitude (Table 1).

It was found for both Scots pine (Table 5) and Norway spruce (Table 12) that seedlings from lower altitudes had a higher content of both chlorophyll and carotenoids than seedlings from higher altitudes.

These results support the findings of Baldwin (1955b) but fail to confirm the results presented by Garret (1969) who reported an opposite relationship between altitude and needle color.

5.3 Influence of Needle Age upon the Content of Needle Pigments

The pigment content in needles of Scots pine is markedly affected by needle age. The older needles consistently have the higher content of chlorophyll (Stålfelt 1927, Langlet 1936, Pravdin 1964, Ollykainen 1967, 1969a) and carotenoids (Kaloudin & Kaloudin 1967, Ollykainen 1967, 1969a).

The results obtained in this report confirm these earlier findings, but current year needles had a higher content of pigments during the first vegetation period when expressed on a dry weight basis due to the lower ratio dry weight:fresh weight for the immature needles (Figures 1 and 4). Calculated on a fresh weight basis the older needles had a higher content of pigments (Figure 2). Older needles also had a higher ratio chlorophyll:carotenoids (Figure 6).

For Norway spruce the results were similar to those for pine, older needles had a higher content of pigments and a higher ratio chlorophyll:carotenoids (Figures 8 and 14). These results are in agreement with the findings of Ollykainen (1970).

The photosynthetic apparatus has the capacity to adjust to light conditions by changing leaf anatomy and/or pigmentation so that available light energy is utilized most efficiently (Berner 1949, Björkman & Holmgren 1963). A low light level can be compensated by an increase of the chlorophyll content in the leaf. This could be one of the reasons why one-year-old needles have a higher content of chlorophyll than current year needles when the content is expressed on a fresh weight or individual needle basis.

In the nursery the seedlings were standing close together (100 plants/m^2) and therefore the older needles were exposed to considerably lower light levels than younger ones due to the shading effect of new shoots.

Carotenoids can function as protective agents against photodestruction of chlorophyll (see review by Krinsky 1968), and it has been suggested from experiments with albino mutants of maize that the amount of chlorophyll will not rise above the level which can be protected by carotenoids (Robertson et al. 1966).

Several authors have reported that both composition and content of carotenoids can be varied by changing the light conditions (Björkman & Holmgren 1963, Lundegårdh 1964, Valadon & Mummery 1969, Öquist 1969). This probably is one of explanations why older needles have a higher ratio chlorophyll : carotenoids than younger needles.

The effect of the variations in the ratio chlorophyll : carotenoids upon photosynthesis in Scots pine needles has been discussed by Linder (1971). It was shown that a higher ratio chlorophyll:carotenoids gave a higher photosynthetic effect in the blue part of the visible spectrum. Consequently, one-year-old needles had a higher photosynthetic effect in blue light than current year needles.

5.4 Influence of Plant Age upon the Content of Needle Pigments

Kaloudin and Kaloudin (1967) have reported that needles from young trees (20 years old) of Scots pine and Norway spruce had a higher content of carotenes than needles from old trees (80 years old).

The present results have shown that when needles of the same age from seedlings of different ages were compared the needles from younger seedlings always had the higher content of pigments when significant differences were obtained (Tables 6—7 and 13).

One-year-old needles from one-year-old seedlings and current year needles from current year seedlings of Scots pine are of juvenile type. Differences in concentration of pigments between these needles and needles of adult type might be due to the different anatomical structure and not to different plant age (Tables 6 and 8).

The difference in pigmentation of needles in relation to plant age might be caused by a sub-optimal supply of fertilizers. If the used amount of fertilizers was sufficient to give an optimal concentration of needle pigments in young seedlings but not in the older ones, the differences found in needle pigmentation could possibly occur. However, it was shown in Table 14 that in spite of a double dose of fertilizers the difference in chlorophyll content related to plant age still existed. Also the relationship between needle age and content of chlorophyll was maintained after extra supply of nutrients.

5.5 Influence of 'Greenhouse Climate' upon the Content of Needle Pigments

The environmental factors differing most between the greenhouse and the open were CO_2-content in the air, air humidity, temperature and light intensity.

CO_2: Partial pressure of CO_2 could be three to four times higher in the greenhouse than what is normal in the air. A CO_2-supplemented atmosphere affects positively the growth of pine seedlings (Sirén

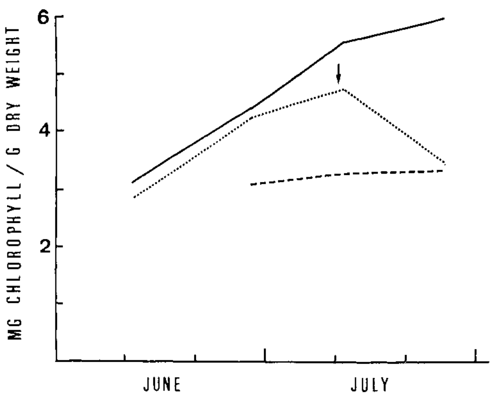

Figure 18 The content of chlorophyll in current year needles of Scots pine seedlings during the period June—July 1968. ---- seedlings grown in a greenhouse with CO_2-supplemented air. —— seedlings grown in a greenhouse without CO_2-enrichment. --- seedlings grown in the open. The arrow indicates the day when the plastic cover was blown away during a storm.

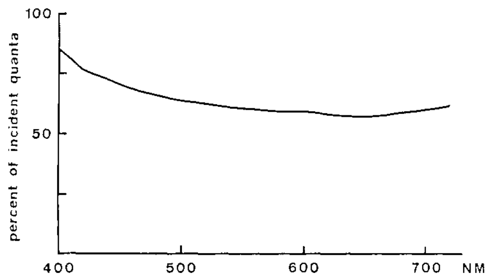

Figure 19 The distribution of quanta, within the visible spectrum, transmitted through the plastic cover of the greenhouse in per cent of incident quanta.

1967, Funsch et al. 1970, Yeatman 1970, Aldén 1971) but did not seem to have any pronounced influence on the pigmentation of the needles (Table 9).

Air humidity: No apparent correlation between the amount of pigments in needles of Scots pine and air humidity has been found (Gerold 1959 b). The design of the present investigation did not make it possible to study the influence of air humidity upon the pigmentation.

Temperature: Low temperatures are known to retard chlorophyll formation but only the temperatures during the light period exert an influence on the synthesis of chlorophyll (Alberda 1969). The very long days during summer in northern Sweden together with the higher temperature in the greenhouse during the light but rather chilly 'nights', could be of considerable advantage for the biosynthesis of chlorophyll in the needles of greenhouse-grown seedlings (Figures 16 and 17).

Little is known about the influence of temperature upon the synthesis of carotenoids. Moster and Quachenbush (1952) found that low temperature did not decrease accumulation of total carotenoids in maize seedlings. The higher temperature in the greenhouse favors therefore chlorophyll synthesis to a greater extent than the synthesis of carotenoids. This resulted in a higher ratio chlorophyll : carotenoids in needles of greenhouse-grown seedlings (Tables 4 and 11).

The effect of the 'greenhouse climate' upon the chlorophyll content in the needles was shown drastically when during a storm the plastic cover from one of the greenhouses was blown away (Figure 18).

Light: On clear days around 30 % of the incident radiation (300—3 000 nm) was absorbed or reflected by the plastic cover of the greenhouse (Backlund & Perttu 1971). Within the visible spectrum (400—730 nm) the plastic cover transmitted around 60 % of the incident light quanta (Figure 19). Berner (1949) reported that a 40 % decrease in light intensity resulted in an increase of chlorophyll in both Scots pine (30 %) and Norway spruce (80 %).

Within the visible spectrum the light was transmitted rather uniformly so there is no reason to expect changes in pigmentation due to the quality of the incoming light.

The effect of high and low light levels upon the pigmentation in needles has already been discussed in connection with the relation between needle age and the content of chlorophyll and carotenoids (Section 5.3.).

6 Conclusions

The results presented show a pronounced seasonal variation of both chlorophyll and carotenoids in the needles of Scots pine and Norway spruce. The content of pigments was influenced also by growing conditions, plant age, needle age and origin of the seed source. Plants grown in the greenhouse had a higher content of chlorophyll and carotenoids than plants grown in the open, due to a higher temperature and a lower light level in the greenhouse. When needles of the same age but from seedlings of different ages were compared the needles from younger seedlings had a higher content of pigments.

Older needles had a higher content of pigments than younger ones. Seedlings from low altitudes had a higher content of both chlorophyll and carotenoids than seedlings from higher altitudes.

The new technique of growing coniferous seedlings in greenhouses during the vegetation period does not have any harmful effect upon the seedlings, as far as the pigmentation of the needles is concerned.

Caution should be exercised whenever the needle color is used as an index of the physiological state of nursery stocks as long as the seasonal and individual variation of pigments in the stock is unknown.

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Sammanfattning

Under det senaste årtiondet har det blivit allt vanligare att driva upp tall- och granplantor i växthus med CO_2-gödsling under delvis kontrollerade odlingsbetingelser. Plantorna odlas i plastväxthus vars plasttäckning på hösten avlägsnas så att plantorna fram till nästa vegetationsperiod står under samma klimatförhållanden som frilandsodlat material. Med denna odlingsteknik erhålles större plantor på kortare tid än vid konventionell frilandsodling.

Det har redan visats att barrträdsplantor växer bättre i växthus med CO_2-gödsling än på friland (Sirén 1967, Funsch et al. 1970, Yeatman 1970, Aldén 1971) men trots detta finns det ett behov av att belysa åtskilliga andra aspekter av den nya odlingstekniken, till exempel:

1. Hur anpassar sig plantorna till växthusmiljön?
2. Hur reagerar plantorna när de klimatiska betingelserna abrupt ändras i och med att plasten tas av växthusen på hösten?
3. Finns det några kvarvarande effekter, positiva eller negativa, av att odla barrträdsplantor i växthus?

För att undersöka vissa av dessa frågor inleddes sommaren 1966 ett omfattande forskningsprogram, vid Institutionen för Skogsföryngring, där olika fysiologiska aspekter på tall- och granplantor odlade under olika plantskolebetingelser studeras (Aldén & Eliasson 1970, Sirén et al. 1970, Aldén 1971, Linder 1971).

Pigmentinnehållet i blad är troligen den mest använda indikatorn på det fysiologiska tillståndet hos plantor. Avvikande färg hos blad eller barr är därför ofta använt som ett symtom på näringsbrist. Nordliga provenienser av tall har emellertid en kraftig årstidsvariation av pigment i barren och denna variation kan ofta påminna om de förändringar som uppkommer på grund av mineralnäringsbrist. Det var därför av speciellt intresse att dels jämföra pigmenteringen hos plantor odlade under skilda betingelser, dels att kartlägga den naturliga årliga variationen av klorofyll och gula pigment (karotenoider) i barr från dessa plantor.

De resultat som framlägges i denna uppsats har erhållits vid Skogshögskolans plantskola i Vidsel (BD-län). Plantmaterialet har utgjorts av tall- och granplantor, vilka odlats antingen i plastväxthus eller på friland.

Resultatet av undersökningen kan kortfattat sammanfattas i nedanstående punkter:

1. En kraftig årstidsvariation av klorofyll och karotenoider hos både tall och gran registrerades. Halten klorofyll varierade mer än halten karotenoider, vilket förorsakade gul vinterfärgning.
2. Några negativa effekter av växthusdriften på barrens pigmentinnehåll kunde ej iakttas, utan växthusplantor hade genomgående högre halt pigment än frilandsodlat material även under de perioder då plantorna var utsatta för samma klimatiska förhållanden som frilandsplantor.
3. Äldre barr hade högre halt klorofyll och karotenoider än yngre barr oberoende av växtplats.
4. Om barr av samma ålder från plantor av olika ålder jämfördes hade barr från yngre plantor högre halt pigment. Denna skillnad kvarstod även vid övergödsling.
5. Barrens pigmentinnehåll var proveniensberoende. Plantor från Zon 0 och 2

(0—100 respektive 200—300 m.ö.h.) hade högre halt pigment än plantor från Zon 3 (300—400 m.ö.h.).

6. Ifall plantans färg användes som ett vitalitetsmått bör försiktighet iakttas så länge som årstids- och individuell variation av klorofyll och karotenoider hos plantmaterialet är okänd.

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