Seasonal Recovery of **CHLOROTIC NEEDLES** *in Scotch Pine*

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The study reported in this paper was done by the author as part of his graduate studies, in cooperation with the Northeastern Forest Experiment Station's project 1502, timber-related crops research, under the direction of Dr. Arnold Krochmal, project leader. The project, with headquarters at Berea, Kentucky, provided materials, trees, and laboratory facilities for the study.

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DISCOLORED NEEDLES AND CHRISTMAS TREES

SCOTCH PINE, which is planted widely for use as Christmas trees, has an unfortunate tendency to discolor during the winter months. The needles of many trees turn yellow just at the time when rich green foliage is wanted. This, of course, is of great concern to Christmas tree growers.

As part of a research project on Christmas trees being carried on by the USDA Forest Service's Northeastern Forest Experiment Station, the author made a cooperative study of how discolored needles recover their normal color in February and April. Though this does not solve the Christmas tree growers' problem, it does shed some light on the process involved in the discoloration.

The study confirmed that the seasonal yellowing of the needles is due primarily to the loss of chlorophyll and, conversely, that a sharp increase in chlorophyll concentration accompanies the return to summer green.

BACKGROUND

When a plant exhibits unusual coloration, a nutrient deficiency is usually suspected. If with Scotch pine (*Pinus sylvestris* L.), a mineral deficiency is responsible for the discoloration, why does the discoloration take place only during the dormant season, and to different degrees among different races at the same site?

Gerhold (*1959a*) studied nine foliar elements in green and chlorotic needles collected in August and February from six races of Scotch pine, but could establish no connection between pigments and elements to account for any appreciable amount of discoloration.

Later Gerhold (*1959b*) established that short photoperiods, intense sunlight, and low temperatures are required for the discoloration to occur. He could not attribute any effect to relative humidity.

White and Wright (*1966*) used growth chambers to study the reverse change—from yellow to summer green. They found that branches maintained at a daytime temperature of 17°C. or higher regained color in a few days. High temperatures in the absence of light did not result in a color change, and the rate of change was affected very little by night temperature.

The pattern of discoloration is genetically fixed and does not change as the tree grows older. Dengler (*1938*) and Münch (*1924*) had shown earlier that needle color characteristics are indeed inherited by second-generation progeny on a foreign site, although site factors do exert some influence on phenotypic needle color (*Gerhold 1959a*).

MATERIALS AND METHODS

Three races of Scotch pine were selected for study solely on the basis of needle color. Two of these races (Spanish and Austrian) were grown in an outdoor plantation; and the third race (French) was grown in an environmental control chamber. The chloroplast pigments and the foliar nitrogen concentrations were determined for all three races.

Trees Grown in Plantation

Trees of the Spanish and Austrian races were growing in the 5-year-old plantations maintained by the USDA Northeastern Forest Experiment Station on the Berea College Forest, at Berea, Kentucky. Elevation was about 950 feet.

Six trees of each race were marked, and needle samples were collected six times from each tree in the periods from mid-February to early March and from Mid-April to early May. Fifteen needles of the current year's foliage were picked from the lateral branches on the south side of each tree, and were placed in vials.

Ten needles were taken from each vial. Those from tree 1 and tree 2 were placed together, and those from tree 3 and tree 4 were placed together, and so on. The combined total of 20 needles were then wrapped loosely in cheesecloth and placed in a −5°C. freezer for analysis of chloroplast pigment later. Five needles from each of the same pairs of trees were cleaned and placed in uncapped vials, which were then placed in an oven and dried at 65°C. for 2 weeks.

Trees Grown in Environmental Chamber

Two-year-old seedlings of the French race of Scotch pine were potted in sandy loam soil, using clay pots. Four seedlings were placed in a Sherer environmental chamber.

The seedlings were first subjected to a spring-like environment for 2 weeks. The temperature was maintained at 21°C. during the day and 10°C. during the night. Day-length was set at 14 hours, with the incandescent lights burning from 6 a.m. till 8 p.m. and the fluorescent lights burning from 11 a.m. till 3 p.m. At the end of 2 weeks a needle collection was made and handled in the same manner mentioned earlier.

After the first collection, the temperature was set at 40°C. during the day and 1.67°C. at night. Day-length was set at 10 hours, with the incandescent lights burning from 7 a.m. till 5 p.m. and the fluorescent lights burning from 1 p.m. till 3 p.m. After 3 weeks, when discoloration seemed to be at its maximum, a second collection was made.

After the second collection was made, the temperature and

photo-period were adjusted to simulate Kentucky conditions in April. The temperature was maintained at 21°C. during the day and 10°C. at night. Day-length was set at 14 hours, with the incandescent lights burning from 6 a.m. till 8 p.m. and the fluorescent lights burning from 11 a.m. till 3 p.m. In about 10 days, after the needles had regained their color, a final collection was made.

Chloroplast Pigment Determinations

The method of extraction and separation followed closely that of Schertz (*1928*). The concentration of pigments in solution was determined from absorption measurements taken with a colorimeter. Chlorophyll a was determined from the absorption at 663 mμ. and chlorophyll b from the absorption at 645 mμ.

Five-ml. aliquots of the acetone solution were then air-dried and the pigment residues were resuspended in 5 ml. petroleum ether for total carotenoid determination. Flavanoids were washed out of the ether solution with 5 ml. of 1-percent sodium carbonate solution, and chlorophylls were removed with methanolic potassium hydroxide (Snell *1967,* vol. 3a, pp. 24-31).

The following formulas derived by Gerhold (*1959a*) and Arnon (*1949*) were used:

Chlorophyll-a, grams per liter = 0.0127 (663) — 0.00269 (645)
Chlorophyll-b, grams per liter = 0.0229 (645) — 0.00468 (663)
Total carotenoids, mols per liter = 451/132, 590

Nitrogen Determination

For the determination of nitrogen, the Kjeldahl method was employed as outlined by Kabat and Mayer (*1961*) and C. Yang (personal communication, 1970)

RESULTS AND DISCUSSION

Chloroplast Pigments

It had been assumed previously, and demonstrated by Gerhold (1959a), that a decrease in chlorophyll accompanied by an increase in carotenoids is primarily responsible for the winter discoloration observed in the needles of Scotch pine. The results obtained in my study show an inverse relationship. My study dealt

with the changes in discolored needles as they convert from yellow to green, whereas Gerhold investigated the changes from green to yellow.

The Spanish race, the only one of the three that kept a good winter color, had the highest average concentration of chlorophylls a and b during the February-March period (table 1). The French

Table 1.—*Average chloroplast pigment content of current year's needles in three geographic races of Scotch pine*

[In grams per liter]

Date	Race	Chlorophyll		Total carotenoids
		a	b	
16 Feb 1970	Spanish	0.0554	0.0197	0.0294
	Austrian	.0439	.0155	.0188
21 Feb 1970	Spanish	.0551	.0219	.0450
	Austrian	.0503	.0195	.0173
24 Feb 1970	Spanish	.0882	.0343	.0444
	Austrian	.0653	.0201	.0448
28 Feb 1970	Spanish	.0507	.0241	.0629
	Austrian	.0308	.0355	.0486
8 Mar 1970	Spanish	.0586	.0200	.0394
	Austrian	.0329	.0205	.0344
10 Mar 1970	Spanish	.0725	.0199	.0359
	Austrian	.0545	.0183	.0219
6 Apr 1970	Spanish	0.0653	0.0235	0.0332
	Austrian	.0787	.0278	.0215
11 Apr 1970	Spanish	.0665	.0213	.0301
	Austrian	.0654	.0288	.0577
15 Apr 1970	Spanish	.0630	.0298	.0415
	Austrian	.0689	.0203	.0321
18 Apr 1970	Spanish	.0727	.0266	.0288
	Austrian	.0736	.0348	.0277
26 Apr 1970	Spanish	.0688	.0300	.0371
	Austrian	.0720	.0254	.0181
3 May 1970	Spanish	.0840	.0381	.0297
	Austrian	.0957	.0447	.0217
April	French	0.1094	0.0469	0.0238
February	French	.0364	.02088	.0267
April	French	.1317	.0689	.0235

and Austrian races, which were noticeably discolored, both increased their chlorophyll-a concentrations by over 200 percent. They also increased chlorophyll b: the Austrian race by 81 percent and the French race by 177 percent as their needles regained their summer color.

These findings provide a basis for stating that a reduction in total chlorophyll content in the needles of Scotch pine is primarily responsible for the discoloration of the needles in many races of Scotch pine during the winter months. And conversely, the sharp increase in total chlorophyll is responsible for the change from winter yellow to summer green.

Gerhold (*1959a*) found that the changes in carotenoid concentrations did not parallel changes in needle color, but did increase at about the same rate from August to February in races that discolor and those that remain green. He concluded that the carotenoids contribute to needle color, but that the primary cause of discoloration is the unmasking of the carotenoids by a sharp reduction in chlorophyll.

The Austrian race had an average of 55 percent less carotenoid concentration than the Spanish race in the February-March period, and an average of 31 percent less in the April-May period. The French race had an average of 42 percent less than the Spanish race during the February-March period and an average of 38 percent less during the April-May period. I found the average carotenoid concentrations in February-March were higher in the race that did not discolor than in the two races that did discolor.

This confirmed the basic assumption that a reduction in chlorophyll concentration rather than an increase in carotenoids is the major cause of discoloration.

Foliar Nitrogen

The fluctuation of chlorophyll content having been directly linked with the changes in needle color in Scotch pine gives additional assurance for reasoning further that if any nutrient element is involved in discoloration, it must bear a relationship to the chloroplast pigments. In this study, foliar nitrogen, which is essential for chlorophyll synthesis, was chosen as the element to be studied.

If a deficiency in nitrogen were responsible or partly responsible for the seasonal discoloration, then one would expect the nitrogen concentration to be lower in February in those races that discolor than in those that remain green. The results obtained in my study did not substantiate this assumption. Rather, the race that discolored the most (Austrian) had a 21-percent higher foliar nitrogen concentration in the February-March period than did the Spanish race (table 2), and maintained a 22-percent higher

Table 2.—*Average nitrogen content of current year's needles in three geographic races of Scotch pine*

Date	Race	Percent dry weight of nitrogen
16 Feb 1970	Spanish	0.73
	Austrian	1.04
21 Feb 1970	Spanish	1.19
	Austrian	1.27
24 Feb 1970	Spanish	.90
	Austrian	1.22
28 Feb 1970	Spanish	.85
	Austrian	1.27
8 Mar 1970	Spanish	.86
	Austrian	.99
15 Mar 1970	Spanish	.95
	Austrian	1.30
6 Apr 1970	Spanish	1.00
	Austrian	1.11
11 Apr 1970	Spanish	.94
	Austrian	1.03
15 Apr 1970	Spanish	.79
18 Apr 1970	Spanish	.69
	Austrian	.84
26 Apr 1970	Spanish	.77
	Austrian	.94
3 May 1970	Spanish	.75
	Austrian	.94
April	French	0.81
February	French	.92
April	French	.83

concentration when both races were green during the April-May period.

The French race, which did not discolor as noticeably as the Austrian race because a temperature below 1.67°C. could not be maintained in the environmental chamber, had a 3-percent higher foliar nitrogen concentration than the Spanish race during the April-May period.

In conclusion, no substantial evidence was found to account for any appreciable amount of discoloration due to a nitrogen deficiency. The ranges of nitrogen in the needles can be regarded as falling within the normal variability in Scotch pine.

SUMMARY AND CONCLUSIONS

Chloroplast pigments and nitrogen in the needles of three geographic races of Scotch pine were analyzed and related to seasonal discoloration. No attempt was made to determine why the chloroplast pigments fluctuate from one season to the next; rather the scope of this study was limited to determining foliar concentrations during February and March and comparing them with those of April and May. Chlorophylls a and b and total carotenoids were determined by spectrophotometry, and nitrogen was determined chemically.

Discoloration is caused primarily by the loss of chlorophyll, accompanied by an increase in carotenoid concentration. The needles regain their color when the process is reversed in the spring. In February, total chlorophyll was higher in the Spanish race than in the Austrian and French races, but in April the opposite was true. Carotenoids were higher in February than in April in all three races. No substantial evidence was found to account for any appreciable amount of discoloration due to a nitrogen deficiency.

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