# PART D

# CHEMICAL TECHNIQUES

# CHLOROPHYLL AS AN INDICATOR OF NITROGEN STATUS OF CONIFEROUS SEEDLINGS

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## ABSTRACT

Some problems involved in using the colour of leaves as an indicator of plant condition are discussed in the paper.

Results are presented demonstrating that the chlorophyll content of conifer seedlings differs due to species, needle age, nursery treatment and time of the year. Similar results are shown for a 20-year-old stand of Scots pine. The non-specific feature of chlorophyll variation in leaves makes leaf colour unsuitable as a general indicator of plant condition.

On each sampling occasion there was a strong relationship between the concentration of chlorophyll and that of nitrogen in the needles. The ratio chlorophyll/nitrogen changed between the different sampling occasions, primarily as an effect of the seasonal variation in chlorophyll concentration. To be able to use chlorophyll content in leaves as an indirect measure of the nitrogen content, keys must be worked out for relevant time-spans. Each species and provenance within the stock will need its own key.

Caution should be exercised when using the colour of the leaves as an indicator of plant condition, unless good keys are available for the "normal" variation of chlorophyll in the stock.

## INTRODUCTION

Green colour of plant leaves is the most widely used index to indicate the "vitality" or "condition" of plants. To most people dark green means healthy plants and pale green unhealthy. Unusual coloration of leaves may often, but not always, be caused by nutrient deficiency. For many nursery stocks, keys are worked out using colour charts (Wilde and Voigt, 1952) or colour photos (Benzian, 1965) to help identify deficiency diseases. The problem when using visual methods is that the result is subjective and depends on the experience of the person making the observations. When modifying nursery practice past experience may be inadequate, making recalibration by "trial and error" necessary.

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An objective way of assessing the colour of foliage is to extract the leaf pigments in a solvent and then measure the amount of pigment present in the solution. Simple methods for chlorophyll determination are now available and can be used in almost any nursery (cf. Linder, 1974) where the appropriate instrumentation is available.

Cholorphyll analyses of coniferous nursery stocks reported previously (cf. Linder, 1972) have shown that the chlorophyll concentration in needles varies with species, provenance within species, time of the year, growing conditions, etc. This means that chlorophyll is a non-specific indicator of plant condition and is affected by many abiotic and biotic factors.

The potential for using pigment analyses to detect the effect of air pollution on plants has been discussed by Arndt (1971).

After investigating the variation of chlorophyll in the foliage of *Pinus radiata* D.Don, Wood and Bachelard (1969) concluded that the prospects for using chorophyll as an index of "physiological health" of a tree are limited. A different opinion was expressed by Heinze and Fiedler (1976) who considered that chlorophyll content was a simple measure by which to assess site quality, growth performance, and nitrogen status of trees.

The present paper is an attempt to illustrate the usefulness and the problems associated with using chlorophyll content as an indicator of condition and/or nitrogen status in conifers.

## MATERIAL AND METHODS

### *Plant Material and Sampling*

Nursery studies: The plants were grown in a research nursery in northern Sweden (65° 48′ N; 20° 34′ E; alt. 80 m). Seedlings were grown either in plastic greenhouses (May-August) or out-doors. All seedlings were treated in the same manner with respect to water, fertilisation, fungicides, insecticides, etc. The plant material presented in this report consisted of two- and three-year-old seedlings of Scots pine (*Pinus silvestris* L.), Norway spruce (*Picea abies* (L.) Karst.) and black spruce (*Picea mariana* Mill.). More details about the plant material and the growing conditions can be found in Linder (1972).

The needles from 10 randomly-chosen seedlings were used for each sample. Current and one-year-old needles from the terminal leaders were used for the analysis and all samples were processed on the day of collection. During the period between sampling and extraction of pigments the samples were stored in darkness at +4°C.

Field study: The experimental stand is located in part of the research area within the Swedish Coniferous Forest Project (SWECON) which is situated in central Sweden (60° 49′ N; 16° 30′ E; alt. 180 m). The stand consists of approximately 20-year-old Scots pine and has been subjected to an irrigation and fertilisation experiment since 1974. A detailed description of the stand structure can be found in Flower-Ellis *et al.* (1976), and the experiment is described in Aronsson *et al.* (1977).

Needles were sampled from the irrigation-fertilisation experiment throughout the year from 1974-1979. During the period of vegetative growth the sampling was weekly, but it was less frequent during other parts of the year. Results presented in this report are for 1974 and 1975 only. Needles were collected from the third whorl on first order branches, and all age classes of needles were sampled separately on each occasion. The sample was divided into four sub-samples and one portion was used for the determination of chlorophyll. Other portions were used for growth analyses, carbohydrate concentrations, and concentration of mineral nutrients. Only results concerning chlorophyll and nitrogen will be presented here. For more details see Aronsson *et al.* (1977). All needles used for chemical analyses were frozen in liquid nitrogen and stored in plastic bags at —20°C until processed.

*Analysis of Plant Material*

Each sample was cut into small pieces and carefully mixed. From the mixed sample two sub-samples were taken, one for determination of dry weight and the other for extraction of pigments.

The needle pieces were ground in 100% acetone. The suspension was then filtered through a glass filter and diluted to exact volume. To avoid possible destruction of the pigments by photo-oxidation, all extractions were done in dim dark-room light. In the nursery studies the extracts were stored in darkness at +4°C until measured in a spectrophotometer (Shimadzo MPL-50L). The amounts of chlorophylls and carotenoids were then determined from the formulae given by Holm (1954). More details concerning the sampling and processing can be found in Linder (1972).

In the SWECON study the routines were the same, except that the amounts of chlorophyll were determined by using a "Chlorophyllometer" (AB Ljungberg, Sweden). The instrument is a photometer calibrated for the determination of chlorophylls (a + b) in 100% acetone (cf. Linder, 1974).

Total-N was determined by a micro-Kjeldal method (cf. Aronsson *et al.*, 1977).

## RESULTS AND DISCUSSION

*Nursery Studies*

In the nursery studies it was found that not only did the chlorophyll concentration in needles vary during the season, but it was also dependent on growing conditions, needle age and species (Fig. 1). Plants grown under a plastic cover had a higher concentration of chlorophyll than plants grown outdoors. This effect has been reported earlier by Bourdeau (1959) and Linder (1971, 1972). Berner (1949), reported that a 40% reduction in light intensity resulted in an increase of chlorophyll in coniferous seedlings and Alberda (1969) reported that low temperature during the light period will retard chlorophyll formation. Thus it seems likely that the increase in chlorophyll concentration in greenhouse-grown seedlings was an effect of lower light level and a higher day temperature in the greenhouse than outside. However, since there is a strong relationship between the concentration of chlorophyll and total nitrogen in needles of conifer seedlings (Mergen, 1953; Keller and Wehrmann, 1963) the differences in seedling colour obtained could well have been due to differences in nitrogen status, since the seedlings were given the same fertiliser treatment in spite of different growth rates. When calculating the ratio chlorophyll/nitrogen (Chl/N) (Fig. 2) for seedlings from the two growing conditions the "greenhouse effect" was still well marked and could not be explained by differences in nitrogen status. During periods when all seedlings were grown under out-door conditions a strong positive correlation was found between chlorophyll and the nitrogen concentration of the needles (Fig. 3). As

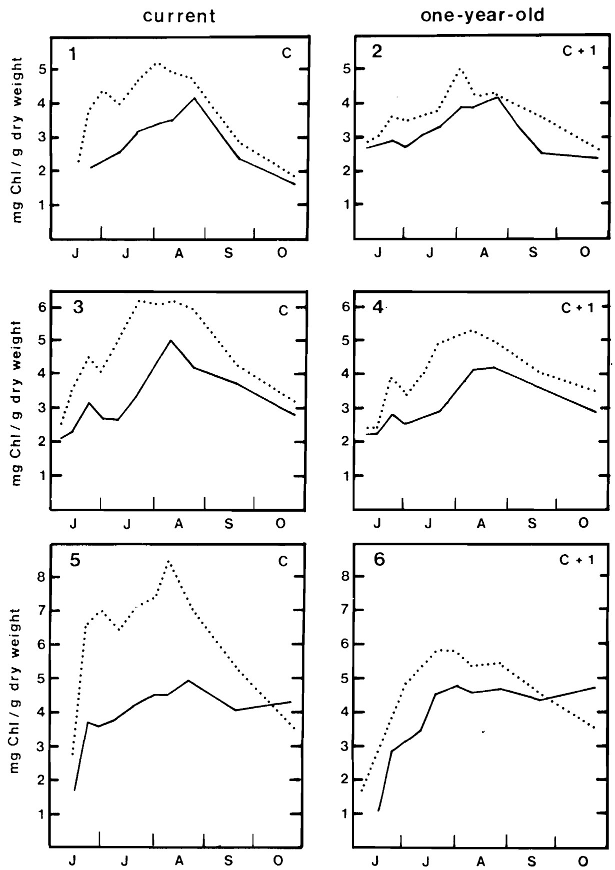


FIG. 1—Seasonal variation in chlorophyll concentration in needles from Scots pine (1, 2), Norway spruce (3, 4) and black spruce (5, 6) seedlings grown inside plastic greenhouses (dotted line) or in the open (solid line). Diagrams on left give the values of current needles (C) and on the right the values of one-year-old needles (C + 1). The plastic cover was on the greenhouses from the beginning of June until the middle of August. The results are for 1969.

an effect of the seasonal variation in both chlorophyll and nitrogen concentrations, the relationship was strongly dependent upon time of the year. Under controlled environmental conditions the relationship between chlorophyll and nitrogen is so stable that once a simple linear regression equation has been worked out a chlorophyll analysis can give a quick estimate of nitrogen concentration (cf. Linder and Ingestad, 1977). However, a specific linear regression equation is necessary for each species and combination of environmental factors.

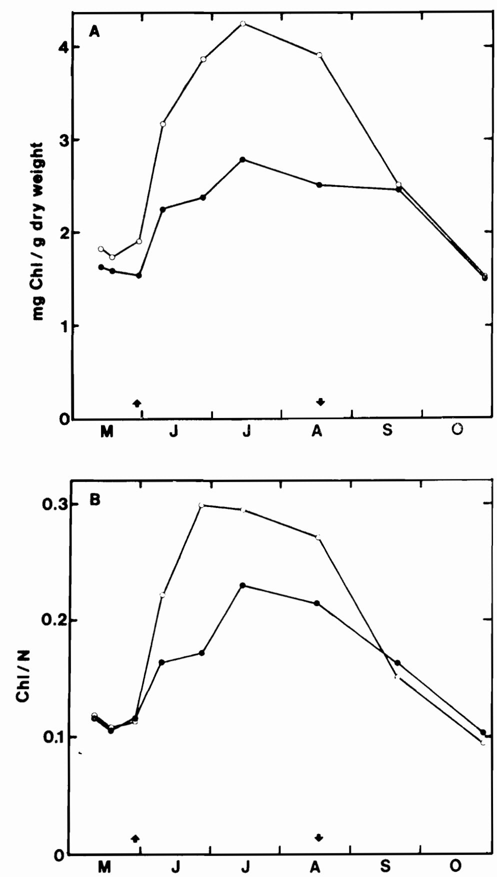


FIG. 2—Seasonal variation in (A) chlorophyll concentration and (B) the ratio chlorophyll/nitrogen (weight/weight) in needles of Scots pine seedlings grown inside plastic greenhouse (open circles) or in the open (filled circles). The arrows indicate when the plastic cover was put on and taken off the greenhouse. The results are for 1970.

## *Field Study*

It was of interest to see whether similar "stable" relationships exist under natural environmental conditions, or if the seasonal fluctuations and year-to-year variability are too great. Since data on needle concentrations of nitrogen in seedlings were available for one season only, this part of the analysis had to be done on data from a young stand of Scots pine. Since the experimental stand had been subjected to an irrigation-fertilisation experiment this gave a wide range of both chlorophyll and nitrogen concentrations (cf. Aronsson *et al.* 1977).

The chlorophyll concentrations found in needles of young trees were lower than for seedlings in the nursery (cf. Figs. 1 & 4). Needles from fertilised trees had a much higher concentration of chlorophyll than needles from untreated trees, but both needle

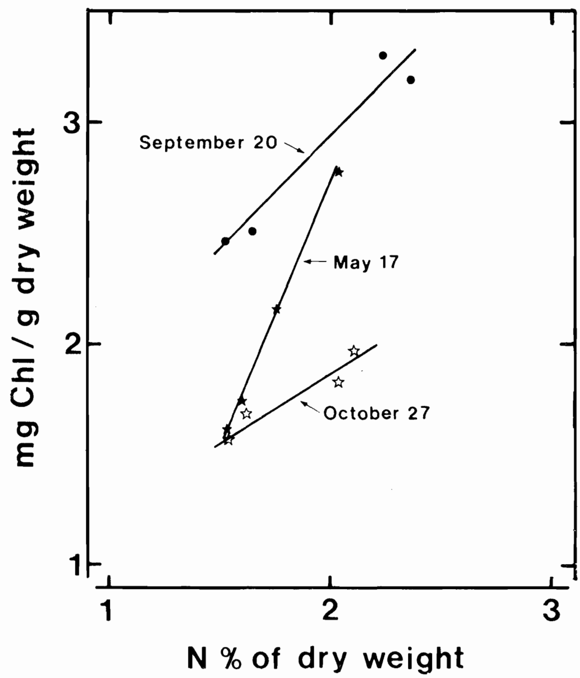


FIG. 3—The relationship between needle concentration of chlorophyll and nitrogen in one-year-old needles of Scots pine seedlings.

categories exhibited a pronounced seasonal fluctuation with a maximum in early August (Fig. 4). This is in agreement with the findings of Pravdin (1964) and Ollykainen (1967). However, if the chlorophyll concentration was expressed as a fraction of foliar nitrogen concentration both seasonal trends and values of fertilised and untreated trees were comparable (Fig. 5). In Fig. 5 the quotients calculated for the growing season of 1975 are compared with those for 1974. For fertilised trees, the quotients were calculated from the time when the treatment started (12 July 1974). Although the seasonal trend was fairly similar in both years the ratios on most dates were lower during 1974. The year-to-year differences recorded were probably an effect of variations in seasonal weather conditions, especially temperature (Fig. 6) and insolation during the two years. No direct correlation was found between year-to-year differences in temperature and corresponding differences in the chlorophyll/nitrogen ratio. This might be because the rate-limiting effect which cool day temperatures have on the synthesis of chlorophyll (Alberda, 1969) is offset by the reduced degradation of chlorophyll through photo-oxidation (Linder, 1971) during dull weather. Since cold weather in summer time often coincides with rainy weather, both production and destruction of chlorophyll will be retarded by low temperature and reduced insolation respectively. More data are needed to analyse the interaction of these effects.

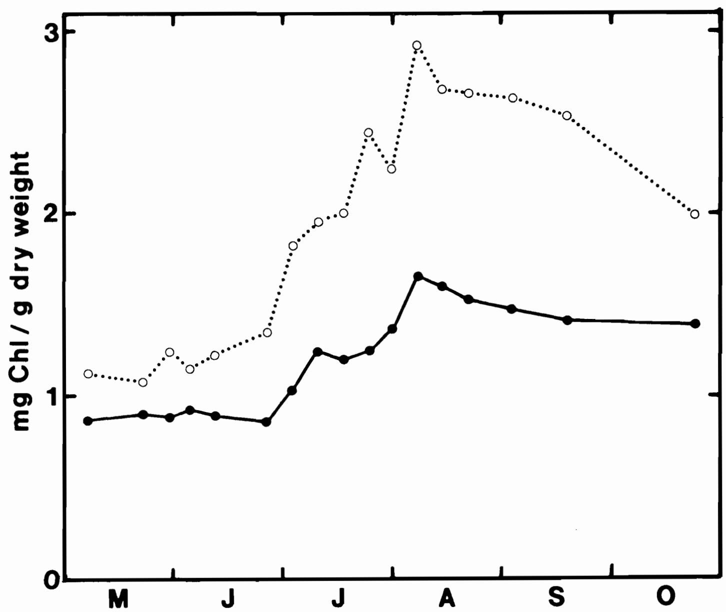


FIG. 4—The seasonal variation in chlorophyll concentration of one-year-old needles of Scots pine. The needles were collected from the third whorl of 20-year-old trees. Untreated plot: solid line; irrigated-fertilised plot: dotted line. The results are for 1975.

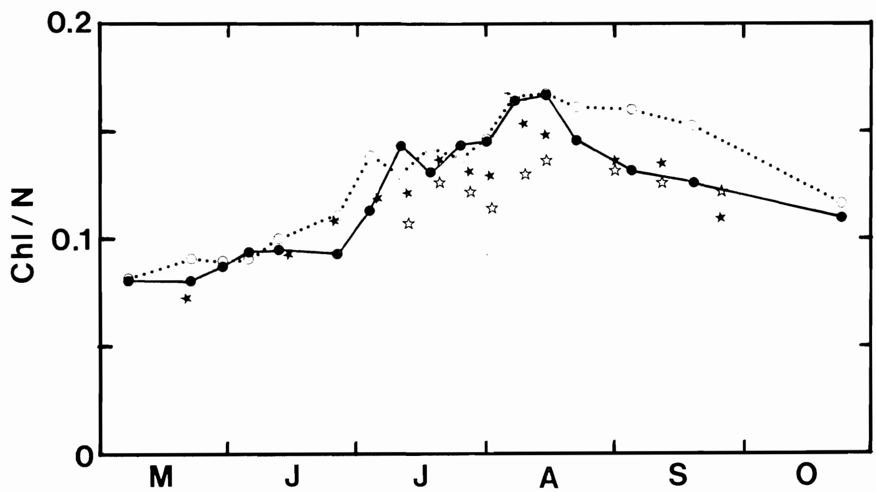


FIG. 5—The seasonal variation of the ratio chlorophyll/nitrogen (weight/weight) in one-year-old needles of Scots pine. The needles were collected from the third whorl of 20-year-old trees. Untreated plot: solid line: irrigated-fertilised plot: dotted line. The curves give the values for 1975 and the stars indicate the ratios found on untreated (filled) and irrigated-fertilised (open) plots during 1974. The treatment started 12 July 1974.

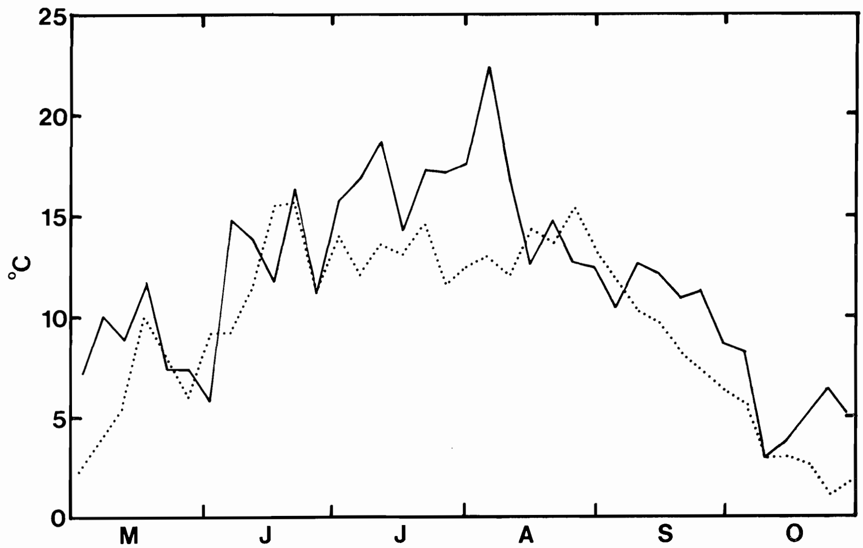


FIG. 6—The average air temperature at the experimental site during the growing seasons of 1974 (dotted line) and 1975 (solid line). The values given are the average value per five-day period.

White and Wright (1967) reported that a colour change in needles of Scots pine became apparent within a few days when twigs were brought from winter conditions to a controlled "spring weather". Their findings indicate that appreciable changes in needle colour can take place within a short period, and this could lead to some error when trying to use the same quotient Chl/N from year to year. On the other hand, the agreement between the two years was reasonable and shows that with an appropriate regression equation, chlorophyll concentration can be used to give an estimate of nitrogen concentration. This could be useful in a nursery where the same schedule is used from year to year. In most cases, the nursery manager wanting seedlings analysed for nitrogen cannot obtain a quick answer. However, where the relationship between chlorophyll and nitrogen concentration has been established, a good estimate can be obtained in less than 30 minutes, enabling the manager to assess quickly the need for nitrogen fertilisation. The disadvantage is that the ratio has to be interpreted with the help of a number of previously established linear regression equations appropriate to particular dates during the season (Fig. 7). On each data there is a very strong positive correlation between nitrogen concentration and the concentration of chlorophyll, but the slope of this relation changes rapidly, primarily as an effect of seasonal changes in chlorophyll concentration.

The method may have application where a large number of foliage samples of comparable age, taken on the same occasion, require determination of nitrogen. A selection of the samples would then be analysed for both chlorophyll and nitrogen to establish a regression equation so that chlorophyll analyses, which are cheaper and quicker than nitrogen assays, can be used to give estimates of nitrogen for the bulk of the samples.

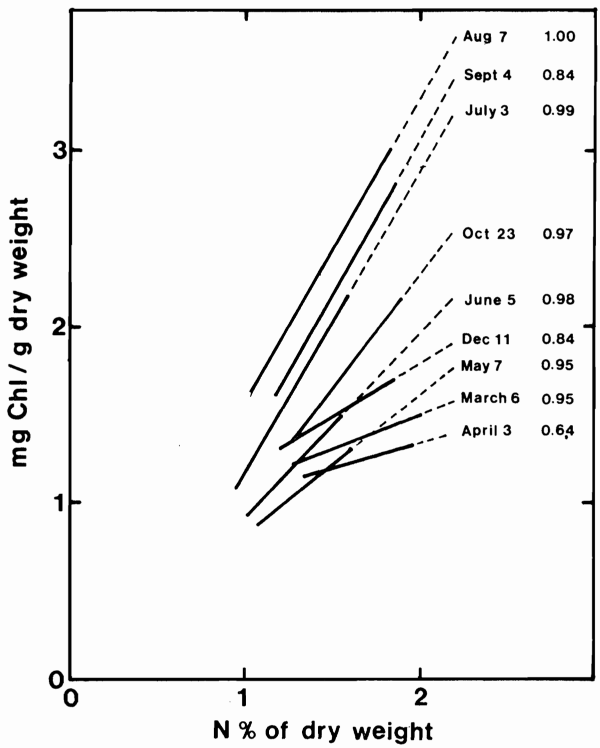


FIG. 7—The relationship between needle concentration of chlorophyll and nitrogen in one-year-old needles of Scots pine during 1975. The solid part of the regression line gives the range of nitrogen content found on each occasion. The sampling date and the coefficient of determination ($r^2$) for each line is given to the right. One sample from each of the four treatments was used for the calculations.

## CONCLUSIONS

The colour of plant foliage is determined by a number of biotic and abiotic factors. Thus, chlorophyll concentration of foliage cannot be used as a general index of nursery stock condition. However, trying to find one single indicator of plant condition is probably as rewarding as trying to find the "philosopher's stone".

Under standardised conditions, simple linear regression equations may be worked out where the relation between chlorophyll concentration of leaves and some other factor is established. By virtue of the strong positive correlation between the concentration of chlorophyll and nitrogen in leaves, chlorophyll analyses can be used to provide quick estimates of nitrogen status of plants. However, previously established regression equations are necessary before the method is of any practical use.

In comparative experimental work, chlorophyll concentration is often a useful indicator for comparing the effect of different treatments.

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