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# Differences in chemical composition of plants grown at constant relative growth rates with stable mineral nutrition

**R.H. Waring[1, 2], A.J.S. McDonald[1], S. Larsson[3], T. Ericsson[1], A. Wiren[1], E. Arwidsson[1], A. Ericsson[4], and T. Lohammar[1]**

[1] Department of Ecology and Environmental Research, Swedish University of Agricultureal Sciences, S-75007 Uppsala, Sweden
[2] Present address: College of Forestry, Oregon State University, Corvallis, OR 97331, USA
[3] Department of Plant and Forest Protection, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden
[4] Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, S-901 83 Umea, Sweden

**Summary.** Leaf chemistry of a willow clone (*Salix aquatica* Smith) differed significantly when grown at constant relative growth rates depending upon the relative availability of nutrients and light. Concentration of amino acids and nitrate were high in plants grown with a relative surplus of nutrients. Concentrations of starch, tannin, and lignin, on the other hand, were high in plants grown with a relative surplus of carbon. Photosynthetic rates, expressed per unit leaf area, were similar when plants were grown under high light conditions, regardless of nutrient availability. Dark respiration was much higher in plants supplied with abundant nutrients than in those with a more limited supply, reflecting differences in nitrogen concentration of the tissue. The experimental approach allows plants to be grown to a standard size with differing, but highly uniform chemistry. Plants grown in such a manner may provide good experimental material to evaluate interactions between herbivores or pathogens and their hosts.

During normal plant development, growth is not constant and chemical composition can be expected to vary, making it difficult, if not impossible, to adequately characterize the qualities of plants that determine their susceptibility to attack by various herbivores and pathogens. In some cases, host plant chemistry can be modified by controlling the environment after all top growth has ceased (Matson and Waring 1984). In many instances, however, it is necessary to assess plant-herbivore and plant-pathogen interactions on growing plants.

From extensive experimental work in mineral nutrition, Ingestad (1979a, 1979b, 1981, 1982) demonstrated that the concentration of nutrient solution could be reduced to very low levels and stable growth rates in the range from 2 to 20% of total dry weight per day could be maintained for seedlings of a variety of hardwood and coniferous species. The essence of Ingestad's approach is that very dilute, but balanced nutrient solutions must be supplied in exponentially increasing amounts each day to exactly meet the nutritional demands of that day's growth. In the analysis of plant tissue, Ingestad (1979a, 1979b) found that the concentrations of mineral nutrients might vary depending upon the rate at which the nutrients were supplied. The concentration and ratios of one nutrient to another in plants grown under a particular environment, however, remained constant over the duration of an experiment.

We reasoned that if plants could be grown with stable mineral nutrient concentrations at constant growth rates then perhaps they could also be grown with stable differences in plant chemistry. We sought to grow plants with stable differences in plant chemistry by controlling the light environment and the nutrient addition rate. We tested this approach by growing cuttings of willow, *Salix aquatica* Smith, under three environments known to provide a range in growth rates and mineral nutrition levels in plant tissue (Ericsson 1981a). The selected environments included: (1) high light with moderate nutrient supply, (2) high light with abundant nutrient supply, and (3) low light with abundant nutrient supply. In this paper we contrast plant performance in the three environments in terms of photosynthesis, dark respiration, carbohydrate allocation, nutrient flux to the leaves, and selected chemical analyses.

## Methods

### *1. Growth of plant material*

Rooted plants were obtained from 5-cm long stem cuttings. During the rooting phase, cuttings were placed in fine sand and allowed to develop under constant light (120 μmol $m^{-2} s^{-1}$), temperature (20° C) and relative humidity (85%). After 10 to 12 days, three sets of 14 plants of similar size were selected and placed in three experimental units in which a dilute (conductivity less than 50 μS $cm^{-1}$), balanced nutrient solution circulated continuously to bathe all roots (Ericsson 1981a). Each unit held 14 plastic vessels of 400 ml volume. The rooting of plants selected to grow at approximately 15% per day was delayed two weeks so that the final size of all plants would not exceed 20 cm in height, a limitation set by the size of chambers available to measure photosynthetic rates. In all treatments a 20-h photoperiod, 20° C air temperature, and 75% relative humidity was maintained throughout the experiments.

*Offprint requests to:* R.H. Waring

The growth rooms were furnished with reflecting walls and an overhead light source of Sylvania F96 T12/GRO/VHO/WS fluorescent tubes with supplemental incandescent lamps providing an irradiance of 310 μmol $m^{-2}$ $s^{-1}$ PAR above the plants. Screening with white cloth reduced the direct irradiance measured to 50 μmol $m^{-2}$ $s^{-1}$ for the low light treatment.

The nutrient supply was unconstrained in two treatments (high light-high nutrients and low light-high nutrients), matching the rate of nutrient uptake possible under the two light regimes (16% and 6% $day^{-1}$), respectively. The nutrient supply was restricted to an increase in supply of 6% $day^{-1}$ for the high light-moderate nutrient treatment.

At up to 5 weekly intervals during the stable growth period, the total fresh weight of each plant was determined and average relative growth rate was calculated. At the end of the experiment, five plants were randomly selected from each treatment for measurement of photosynthesis and dark respiration. During $CO_2$ exchange measurements, shoots of each plant were enclosed in cuvettes in which air flow, temperature, humidity, and light were controlled. $CO_2$ exchange was measured in an open system by infra-red gas analysis (ADC Type 225 MkII) employing a differential mode with a reference gas of 330 ppm $CO_2$. Calculations of $CO_2$ exchange were made according to Sestak et al. (1971), taking into account the calibration temperature of the analyzer.

Following the gas exchange measurements all 42 plants were sectioned into roots, stems, and leaves, the surface area of leaves determined with a Li-Cor surface area meter (Model No. 3310), and the plant parts dried and weighed.

### 2. *Chemical analysis*

Following harvesting, five plants from each treatment were stored at −18° C. Leaves from these plants were later freeze-dried in preparation for assay of tannin content and soluble nitrogen, including amino acids and nitrate. The other nine plants from each treatment were oven-dried at 70° C for 48 h, sectioned, and the leaves were ground through a 0.5 mm mesh in a Culatti mill in preparation for analysis of total nitrogen, starch, and lignin content. Four plants from each treatment were analyzed for nitrogen and 5 for starch and lignin.

Total nitrogen content was determined by micro-Kjeldahl distillation. The fraction of nitrogen in amino acids and in nitrate were determined following extractions with methanol, chloroform, and water (Bielski and Turner 1966). Nitrate was determined from the water extract by spectrophotometry after reduction to nitrite. Nitrogen in the form of amino acids was estimated by subtracting the nitrate content from the total content of nitrogen in the water-phase extract.

Starch analyses were performed after extracting soluble carbohydrates with 80% aqueous ethanol and then treating the residue with 35% aqueous percholoric acid (Hansen and Moller 1975), as modified by Ericsson (1979). The extract was analyzed for starch by the anthrone method. Duplicate analyses were made on each sample.

Tannin content was estimated by the capacity of ground leaves to precipitate hemoglobin from cow blood (Bate-Smith 1973, Martin and Martin 1982). We present the results of these analyses in relative units to the treatment which precipitated the most hemoglobin.

Lignin content was determined by first extracting water and acetone soluble fractions (Berg and Staaf 1980) of the dried residue (Berg et al. 1982).

### 3. *Statistical analyses*

Treatments were compared by one-way analysis of variance. If an F value indicated significant differences among treatments, we calculated least significant differences at $P = 0.05$ and compared means among treatments (Fisher protected analyses in Steel and Torrie 1980).

## Results

The chemical composition of willow leaves grown under the three different environments varied considerably (Table 1). The total N content exceeded 5% of the dry weight in leaf tissue from plants with unconstrained nutrient supply. Differences in specific leaf weight of nearly three-fold (Table 2) resulted in N content per unit area being quite similar for both the treatments growing at approximately 6% $day^{-1}$. Under both high and low light conditions with unconstrained supply of nutrients, amino acids increased significantly in contrast to plants grown with more limited nutrient supply. Moreover, nitrate became detectable when nutrient supply was unconstrained.

The increased nitrogen content in leaves of plants with unconstrained nutrient supply did not improve photosynthetic performance per unit of leaf area. High light, however, increased photosynthetic rates by more than six-fold. Shoot dark respiration was highest in plants growing at approximately 16% $day^{-1}$. Higher nitrogen concentrations appeared to result in a proportional increase in maintenance respiration, as evidenced by the more than 50% difference noted in dark respiration between treatments with similar relative growth rates but with N status differing by nearly 40%.

**Table 1.** Chemical composition of willow leaves (*Salix aquatica*) grown under specified environments at stable growth rates and stable mineral nutrition

| Compound | High light High nutrients | Low light High nutrients | High light Moderate nutrients |
|---|---|---|---|
| Total N % dry wt. | 5.04[a] | 5.11[a] | 1.91[b] |
| Total N mg N $dm^{-2}$ leaf | 21.5[a] | 13.4[b] | 14.0[b] |
| Amino acids mg N $dm^{-2}$ leaf | 2.4[a] | 2.3[a] | 0.9[b] |
| Nitrate mg N $dm^{-2}$ leaf | 1.0[a] | 1.7[b] | 0.0[c] |
| Starch % dry wt. | 5.1[a] | 5.3[a] | 20.7[b] |
| Tannins relative units | 0.65[a] | 0.64[a] | 1.00[b] |
| Lignin % dry wt. | 20.8[a] | 13.4[b] | 24.5[c] |

Values with different superscripts differ significant at $P = 0.05$

**Table 2.** Performance of willow (*Salix aquatica*) under specified environments

| Variable | High light High nutrients | Low light High nutrients | High light Moderate nutrients |
|---|---|---|---|
| Relative growth rate, % $day^{-1}$ | 16.1[a] | 6.8[b] | 5.5[b] |
| Unit leaf rate, mg shoot $dm^{-2}$ leaf $day^{-1}$ | 4.3[a] | 1.3[b] | 3.0[c] |
| Leaf wt.: total wt. | 0.56[a] | 0.53[a] | 0.37[b] |
| Root wt.: total wt. | 0.20[a] | 0.15[b] | 0.38[c] |
| Specific Leaf wt. g $dm^{-2}$ | 0.48[a] | 0.28[b] | 0.80[c] |
| Net photosynthesis mg $CO_2$ g leaf $hr^{-1}$ | 26.2[a] | 6.9[b] | 16.4[c] |
| Net photosynthesis mg $CO_2$ $dm^{-2}$ $hr^{-1}$ | 12.5[a] | 1.9[b] | 13.1[a] |
| Shoot dark resp. mg $CO_2$ $g^{-1}$ $hr^{-1}$ | 5.7[a] | 4.0[b] | 2.1[c] |
| Net leaf carbon flux mg C $dm^{-2}$ leaf $hr^{-1}$ | 1.52[a] | 0.39[b] | 0.90[c] |
| Leaf N flux mg N $dm^{-2}$ leaf $hr^{-1}$ | 0.14[a] | 0.04[b] | 0.03[c] |

Values with different superscripts differ significantly at $P=0.05$

Plants with unconstrained supply of nutrients showed significantly lower leaf starch and tannin concentrations as compared to plants grown in high light with moderate nutrient supply. High light intensity, regardless of the nutrient supply, tended to produce plants with high lignin Specific leaf weight reflects not only structural adaptations to high or low light environments but also differences in the amount of starch, tannin, and lignin in the tissue.

Partitioning of carbohydrates into leaves was favored when nutrient supply was unconstrained. Root growth, on the other hand was accentuated when nutrient supply was constrained. Unit leaf rate, the above-ground production per unit area of foliage, increased primarily as function of the light regime and to a lesser extent with the supply of nutrients.

The net flux of carbon to leaves was calculated after taking into account carbohydrate fluxes to other tissues and respiratary losses, and assuming that leaf tissue is 50% carbon. Nitrogen flux to leaves was calculated assuming the plant relative growth rate was equal to the relative rate of nutrient uptake. The ratio of C flux:N flux was 30 in the high light-moderate nutrient treatment, 10 in the high nutrient treatments.

## Discussion

The evidence that plants grown at stable growth rates and provided with a balanced and proportionally increasing nutrient supply would exhibit different chemical properties is documented in Table 1. The underlying assumption that the same differences in chemistry displayed at harvest also existed at any other sampling date is being evaluated in a separate experiment.

Defensive compounds produced by plants obviously depend to a considerable extent upon the environment under which plants grow. Low light conditions, combined with a high nutrient supply, even when nutrients are in balance, may produce plants with high amino acid concentrations and very low concentrations of various carbon-rich defensive compounds (Bryant et al. 1983; Matson and Waring 1984). On the other hand, some species of plants produce nitrogen-rich defensive compounds, e.g., alkaloids, nonprotein amino acids, and cyanogenic glycosides (Harborne 1982, see also Prudhomme 1983).

In general, we might expect plants with a balanced nutrition, growing at moderately high unit leaf rates to have sufficient carbohydrates and soluble nitrogen resources to construct a variety of defensive compounds preceeding and (or) following attack by insects or pathogens. In a subsequent study, Larsson et al. (in press) repeated the study reported here and found that phenolic glycosides concentrations in *Salix aquatica* leaves was closely correlated with unit leaf rates, being highest in plants grown under high light with unconstrained nutrient supply and lowest in plants grown under low light with unconstrained nutrient supply. Moreover, when a small defoliating beetle (*Galerucella lineola* F.) was allowed free access to all plants, the insects selected those plants grown under low light with unconstrained supply of nutrients.

Although mineral nutrition was balanced in the experiments described in this paper, imbalanced nutrition is the rule in nature, especially where atmospheric enrichment of nitrogen and sulfur is pronounced. The general system developed by Ingestad and his colleagues can also be used to create stable conditions of imbalanced nutrition (Ingestad 1979a; Ingestad 1979b; Ingestad and Lund 1979; Ericsson 1981b).

Once preliminary experiments have determined partitioning coefficients between shoots and roots, it is possible to design experiments where plants may be grown with similar amounts of foliage or roots but with different chemical properties. Because roots are completely exposed to the nutrient solution, they may be easily inoculated with symbiotic or pathogenic organisms and subsequently observed under presumably stable conditions.

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