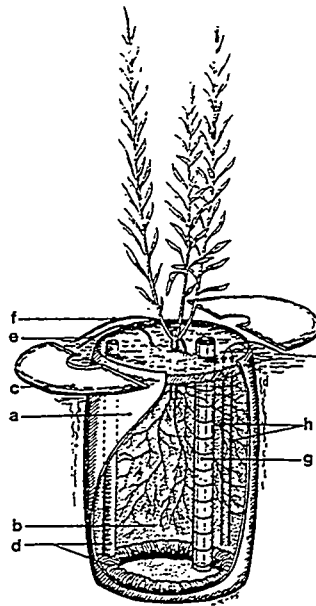




**Growth and biomass distribution in basket  
willow (*Salix viminalis*) in relation to  
water and nutrient availability**

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## Summary

This report describes a study that was made to examine how the production, the period of production and the distribution of biomass in a clone of *Salix viminalis* were affected by shortage of water and nutrients. A field study was performed in which one third of the plants were cultivated under conditions that caused nutrient stress, one third of the plants received too little water and one third of the plants were cultivated under optimal conditions. The experiment lasted for four years including a harvest of the shoots during the autumn of the third year.

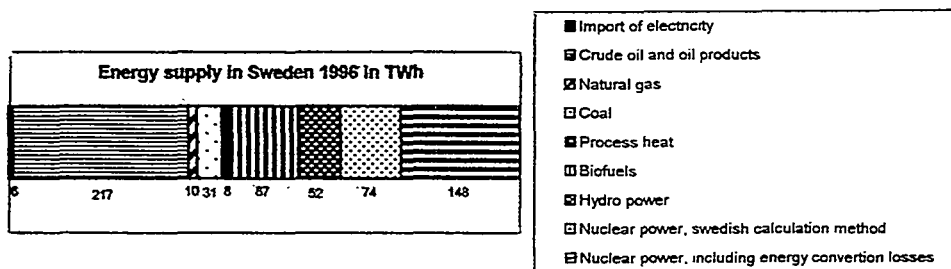
During the period of the experiment the root activity (measured as the number of roots) and the growth of the shoots were measured every second week. The proportion between biomass invested in the roots and in the shoots was measured when plants were harvested at five occasions each year.

Stem production during the third growing season reached 12 tonnes dry matter per hectare and year under non-limiting water and nutrient conditions. Shortage of nutrients and water reduced the rate of stem production to 5 and 2 tonnes dry matter per hectare and year respectively. The root activity did not differ between the treatments. The plants which had full access to water and nutrients allocated less biomass to roots than the stressed plants, 20-30% compared to 30-40% of total biomass.

## Introduction

According to a parliamentary resolution the nuclear power plants in Sweden are to be shut down before the year 2010. The Parliament also has decided that the four rivers which are not used for water power production shall remain as they are. At the 1992 Earth Summit in Rio de Janeiro Sweden declared to keep the emission of carbon dioxide constant until year 2000, and thereafter to reduce the emission.

The sources of energy in Sweden in the year 1996 are shown below (Figure 1):



Source: NUTEK

Sweden is at present facing a difficult problem since it does not seem to be possible to stop the increase in energy consumption and, at the same time, keep the carbon dioxide release constant. In this situation short rotation forestry may contribute to increase of energy availability without increasing emissions of carbon dioxide.

Short rotation forestry can produce a mean of 10 tonnes of dry weight per hectare and year which is equivalent to 4 tonnes of oil or 44 MWh. It is a comparatively cheap way to produce energy. When the cultivation process is fully developed the price is estimated to SEK 0,15 per kWh which can be compared to SEK 0,25 per kWh for oil (Perttu 1985).

Short rotation forestry is based on cultivation of fast growing tree species. Willows, mainly *Salix viminalis*, are the most commonly used species today but trials are also performed with grey alder, birch and poplar. *Salix* can be cultivated on almost all kinds of soils but grows better on soils with a high water content. Geographically the south of Sweden up to Dalälven might be considered suitable. Further north the vegetation period becomes too short. The cultivation is more to be compared to agriculture than to ordinary forestry as it requires the same kind of maintenance. Furthermore the land used for cultivation is tied up for a shorter period. The plants are harvested at intervals of three to five years and new shoots resprout from the stumps. The rotation time for a stand is approximately 25 years, after which the plants are dug up.

Nowadays, when the overproduction of food has forced the farmers to lay their land in fallow, there are large areas which can be used for alternative crops. One of the benefits of producing willow coppice crops instead of conventional forest is that the soil retains its chemical and physical properties, which makes it easier to return to agriculture afterwards.

A *Salix* culture is limed and fertilised and this might affect the environment negatively. Liming, which counteracts the acidification that the plants cause, can be positive if it is performed in the right way. Compared to traditional agriculture the nutrient-leakage would be quite small, since *Salix* is a perennial crop and the roots continue to grow until the soil is frozen (Rytter 1997). Harvest is preferentially accomplished when the soil is frozen. At that time free nutrients already have been taken up by the roots and the accordingly nutrient-rich leaves have fallen to the ground and became litter. This cyclic system probably can maintain with just a little addition of nutrients until a new plant establishment is made (Ericsson 1994).

Cultivation of willow coppice crops might affect the biodiversity of the surrounding flora and fauna negatively. However, properly located plantations can affect the number of species positively (Gurnell *et al.* 1992, Barkham 1992 and Greatorex-Davis *et al.* 1992). Willow coppice crops for example can be used to connect existing areas with deciduous trees in the agricultural landscape. The number of species is favoured if different parts of the plantation consist of different species or clones or even have different intervals between the harvests.

Willow forestry does not necessarily have to make the landscape ugly. If the plantations are made less angular than they usually are today and if they are complemented with groups of bushes and deciduous trees they will be more appealing and a higher biodiversity will be accomplished (Christian 1992).

To develop commercial short rotation forestry to an economically and environmentally acceptable energy source a lot of research at different levels is required. Machines for cultivation and combustion must be improved. Furthermore an increased knowledge of the

physiology of the plant material is required. Based on this knowledge improved plant material can be produced. The plants must have a high growth capacity. This is achieved by a long growth period during which the growth is fast and most of the biomass is stored in the shoots. By experience, we know that the most important factors that affect the production in plants are the supply of water and nutrients.

The experiment, that is evaluated in this report, was started in order to examine how the most commonly used willow species, *Salix viminalis*, responds to shortage of water and nutrients. The project is a part of a comprehensive investigation, with the aim to determine the cultivation demands for the clones that today seem to be the most valuable to cultivate. Based on this information it will be possible to choose the clones that are best in a certain environment. The purpose is that the farmer in the future will be able to calculate the earning capacity of a planned plantation by means of a computer program. This study also aims at getting a greater general understanding of this new crop.

Another part of the project, which is not accounted for in this report, is to examine the water use efficiency of the cultivated clone. This is *inter alia* made through studies of the distribution between the carbon isotopes  $^{13}\text{C}$  and  $^{12}\text{C}$  in leaves. In food crops there is a positive correlation between  $^{13}\text{C}$  and the water use efficiency. Knowledge of this domain also is important to enable the choice of the right clone to a certain area.

In order to create similar growth conditions as in a commercial plantation the plants were placed outdoors in big plastic containers surrounded by plants of the same age. The distance between the plants was the same as in a commercial cultivation and the plants had to compete for light and space. Irrigation and fertilisation were controlled while the other factors, such as climate, were not subjected to deliberate manipulation.

## Materials and methods

### *Materials*

The experimental area is situated in the eastern part of central Sweden in Ultuna close to Uppsala (lat. 59°49'N, long. 17°40'E, 5 m a.s.l.).

Cuttings from basket willows, *Salix viminalis* L., clone 78 183 were planted in spring 1994. In Salixnytt no 2 the clone is described as "a kind with many yellow-greenish and straight stems growing vertically which gives a column like impression". It is also stated that the clone is not very sensitive to frost. To examine how the clone responds to water and nutrient stress the cuttings were placed in three different plots where they were exposed to different treatments. Every cutting was planted in a container of 200 litres (a lysimeter) submerged in the soil. On each plot thirty lysimeters with a spacing of 0.7x0.7 meters were placed. The experiment consisted of a total of 90 lysimeters.

The lysimeters (Figure 2) contained quartz sand, initially almost completely free from humus and nutrients. In the bottom of each container a drainage tube was placed to which a vertical tube was connected. Through this construction excessive water could be removed. Between the cutting and the edge of the lysimeter a transparent plastic tube (a minirhizotron see Figure 3) was put to allow a continuous monitoring of the root activity.

The growth rate of the plants could be controlled and put to a wanted level as the delivery of water and fertiliser was made on daily basis. This was directed from a computer

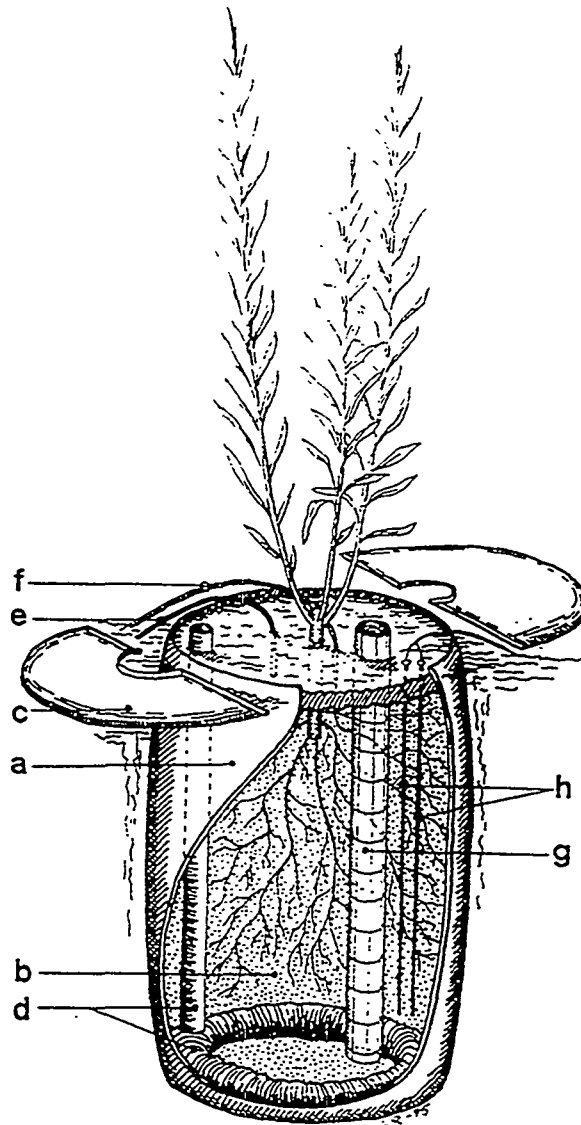
programme. The water potential in the lysimeters was controlled by TDR (Time Domain Reflectance) technique (Topp *et al.* 1980).

The daily nutrient supply was calculated from the growth pattern and the growing capacity of the species. The nutrient solution used is Wallco plant nutrient (Cederroth International AB). Composition of the nutrient solution is given in Table 1.

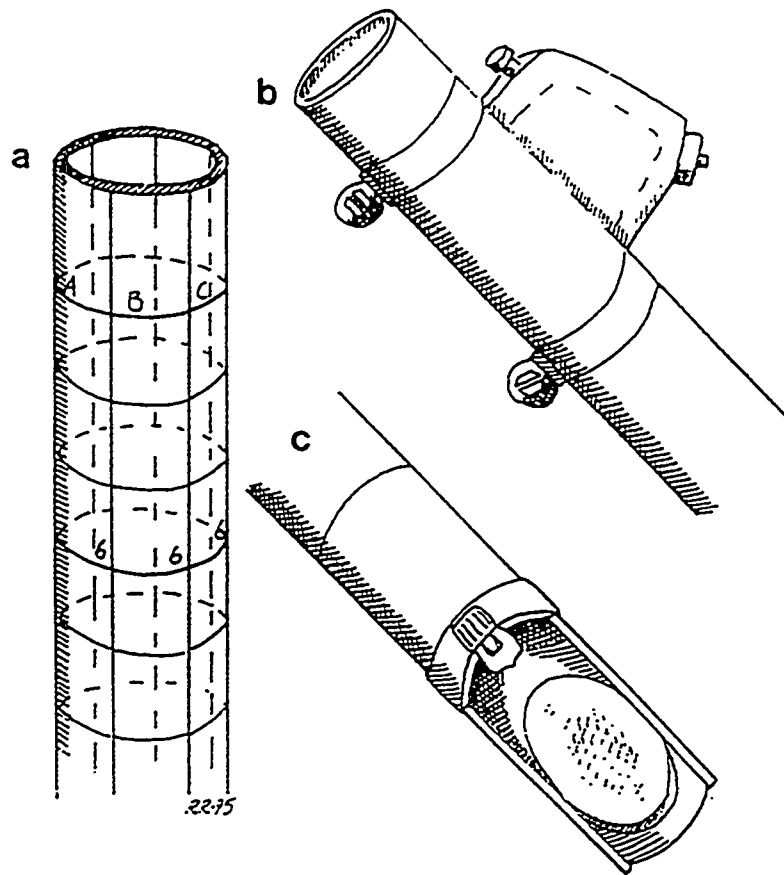
**Table 1.** *The composition of the used nutrient solution given as g/l.*

Amount in g/l	
Macro	Micro
51 g nitrogen in form of	0.17 g iron
20 g ammonium and	0.20 g manganese
31 g nitrate	0.10 g boron
10 g phosphorus	0.03 g zinc
43 g potassium	0.015 g copper
4 g sulphur	0.0004 g molybdenum
3 g calcium	
4 g magnesium	

This mixture reflects the nutrient requirement of *Salix* (Ericsson 1981). During the first year each plant was assumed to use 31 g nitrogen (62 kg/hectare), during the second year 50 g (100 kg/hectare), during the third year 30 g (60 kg/hectare) and during the last year, i.e. the year after the harvest, 25 g (50 kg/hectare).



**Figure 2.** *The lysimeter system developed at the Department of Short Rotation Forestry, SLU, Uppsala,: a. lysimeter consisting of a plastic container with a volume of 200 litres; b. washed quartz sand as growth substrate, with an average grain size of 0,58 mm and free from humus and nutrients; c. a cover in two parts, as protection for precipitation and steam-flow of water, where the central opening is sealed with adhesive mass; d. pipe and drainage tube allowing excessive water to be removed; e. sensor for measurement of soil temperature; f. drip tube for supply of water and liquid fertiliser; g. minirhizotron for studies of fine-root dynamics; h. probes for soil water measurements with the TDR technique (from Rytter 1997).*



**Figure 3.** *The minirhizotrons are made of transparent Plexiglas tubes (fig a). The resolution in space is image frames of 2 cm x 6 cm and each minirhizotron consist of 78 such frames. Grids are painted on the outside of the minirhizotrons using black water-proof ink. The minirhizotrons were installed vertically into holes made with an auger and dry sand was poured into the gaps between the tubes and the sand walls to get smooth contact. The total length of one minirhizotron is 83 cm of which 80 cm is submerged into the sand. The remaining 3 cm of the tube is covered with a lid to prevent roots from daylight exposure. The external tube diameter is 4 cm. Roots growing to the surface of a minirhizotron are counted using an equipment that consist of a plastic tube with a length of 94 cm, a magnifying glass, and a mirror (fig b and c). The mirror is mounted at an angle of 45° and light source is a 10 W lamp placed above the mirror. All image frames of the minirhizotrons are used to get an overall picture of the development of root number during the growing season (from Rytter 1997).*

### *Treatment*

The plants were exposed to three different treatments. Plants cultivated on plot number one were water stressed, the ones on plot number two were nutrient stressed and the plants on plot number three were cultivated under optimal conditions.

During the first year (1994) all plants received water and nutrients in such amounts that the growth was not limited. This was to facilitate the establishment. In the beginning of the second year the plants on plot one got an optimal supply of nutrients and water until the leaves had developed. At that time the water supply was shut off until the plants showed clear signs of water stress. Then the plants were supplied with water again but in so small amounts that the water stress continued. The water content in the sand was kept on a level corresponding to half of the field capacity. When the plants looked really stressed (the leaves of the plants started to turn yellow and fall off) they got an optimal supply of water again so the stand could recover until the next year when the treatment was repeated. The plants on plot two got half of their optimal nutrient supply from the second year and on. The plants on plot three were continuously cultivated under conditions in which the availability of water and nutrients should not limit their growth.

### *Measurements*

Minirhizotrons were placed in six of the lysimeters on each experimental area which makes a total of 18 lysimeters. Through these the amount of roots (number of root tips) was counted every second week during the course of the experiment. On the same plants the stem diameters (at 5 cm in 1994 to 1996 and at about 10 cm in 1997) and the height of the stems were measured every fortnight.

### *Harvests*

During the first year, when the treatments were the same in all of the experimental areas, only three lysimeters from each area were harvested, one in July and two in October. During the following years nine lysimeters from each plot were harvested, one in June, July, August and September and five in October.

The roots were washed clean from sand and sized in fine roots (<1mm) and course roots (>1mm). The dry weight was determined by placing the roots in a drying cabinet at 70°C until the weight was constant, which took about three days.

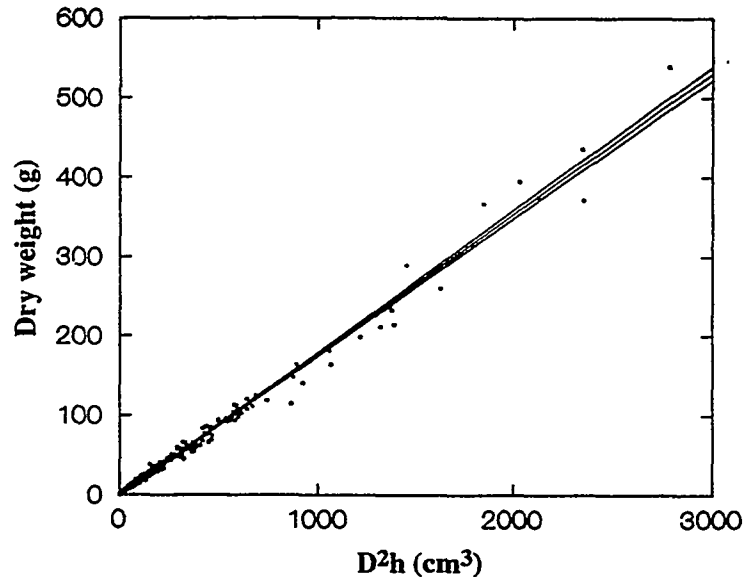
The shoot height and stem diameter were determined. Thereafter the dry weights of leaves and stems were measured after the samples had been dried at 85°C until constant weight, which took about a week.

In the end of 1996 a harvest of all shoots was made and the minirhizotrons were moved to new places. The results from 1997 therefore will be accounted for separately as they are not comparable with the data from previous years.

## Results

### Shoot growth

To get a reliable growth curve the relation between diameter squared times height ( $D^2h$ ) versus dry weight (DW) was examined. For this purpose diameters and heights measured just before harvest and the corresponding dry weights were used. A linear model fitted the data well (Figure 4). The intercept does not differ significantly from zero (which means that one can assume that DW is zero when  $D^2h$  is zero) and the slope of the regression line was  $0.1767 \pm 0.002$ , with an interval of confidence on 95%.



**Figure 4.** Relation between stem dry weight versus diameter squared times height ( $D^2h$ ) in *Salix viminalis*. The 95% confidence interval is indicated around the fitted regression line.

Separate curves were then constructed for each year and treatment. The values of the regression coefficient were in all cases around 0.17 and the intercepts were not significantly separated from zero. The growth will therefore be given as an increase in dry weight calculated through the formula:

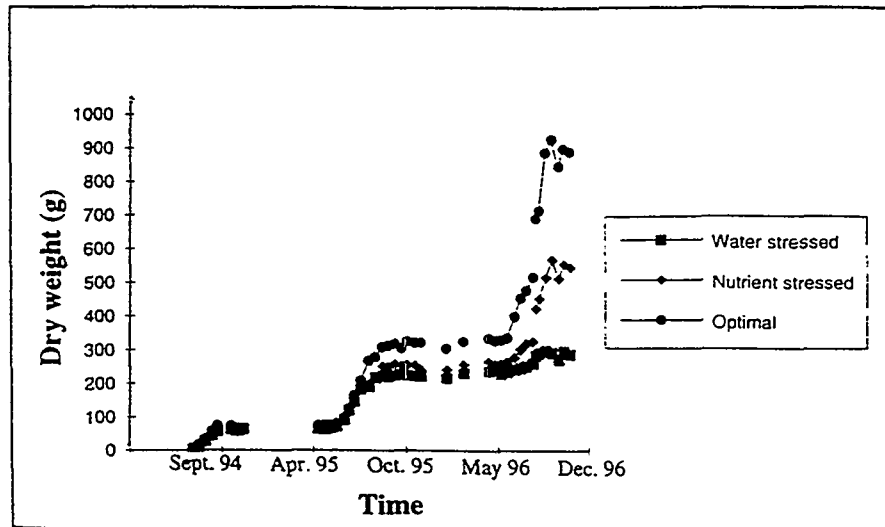
$DW = D^2h \times k$ , where  $k = 0.1767$  and the diameter and the height are the values measured in field.

During the course of the experiment the growth of the plants was determined every second week. Every point in the diagram shows a mean value of the estimated dry weights for the six plants which were analysed at each occasion (Figure 5). During the first year all plants were treated in the same way. The production of each plant was on average about 50 g. The plants which had been cultivated under optimal conditions had a growth of about 300 g during the second year and during the third year the biomass increase was about 600 g per plant. The nutrient stressed plants increased with 200 g the second year and about 250 g the third year and the water stressed plants had the lowest growth with about 150 g the second year and 100 g the third year.

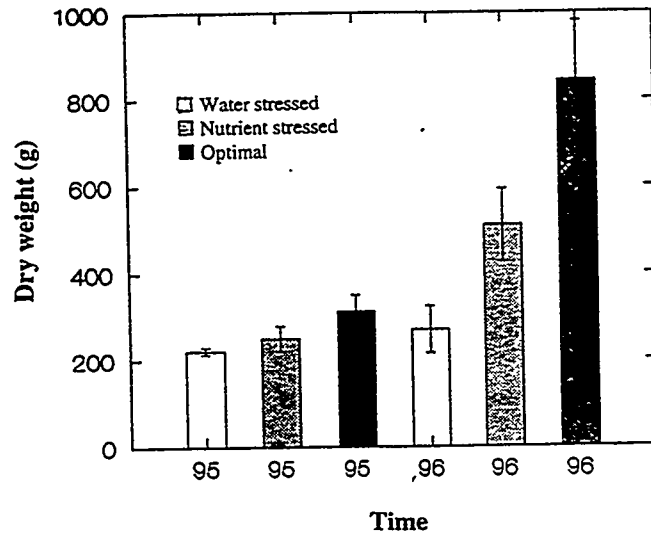
Whether the difference in growth between the plants cultivated on the different areas is significant was estimated at two occasions, in the middle of September year 1995 and at the same time 1996. A mean value was calculated and thereafter a standard error of the mean. The diagram shows that the difference is not significant the second year but the third year (Figure 6).

Irrespective of cultivation conditions all plants started to grow in May. The plants which had been cultivated under optimal conditions and the nutrient stressed ones kept on growing until September while the water stressed plants stopped growing already in August.

The new shoots that came up after the harvest in 1996 responded to water- and nutrient stress in the same way as the older shoots (Figure 7).



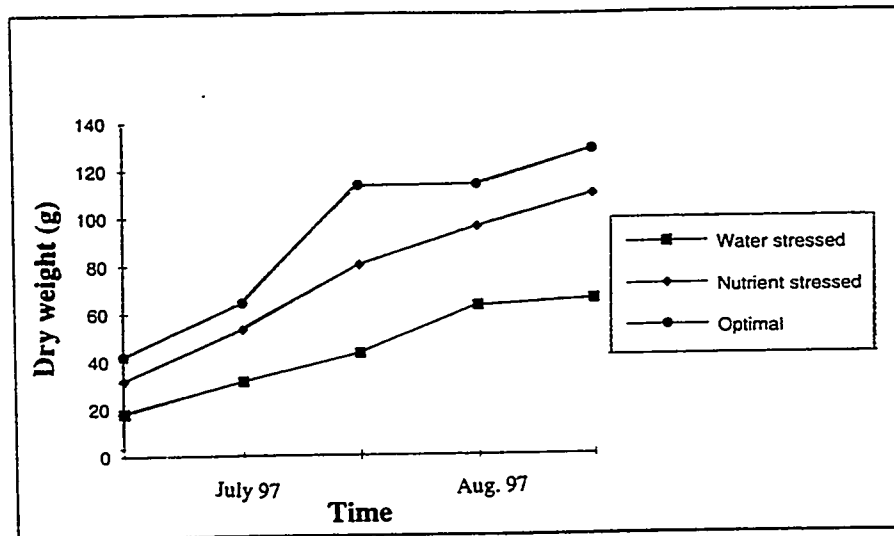
**Figure 5.** Stem production in *Salix viminalis* during the first rotation, from 1994 to 1996 (the time before the harvest of all shoots). Each point represents a mean value of six measurements.



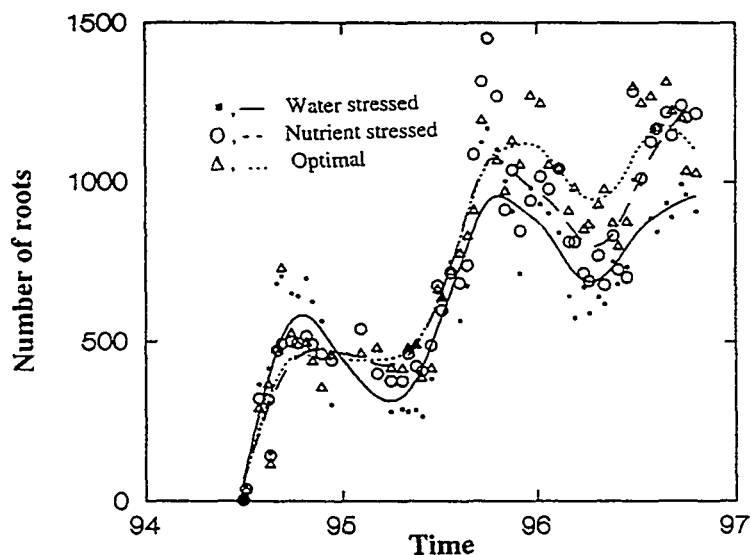
**Figure 6.** Above ground woody biomass in water and nutrient stressed and unstressed plants of *Salix viminalis* in September 95 (first three bars) and September 1996 (last three bars). All bars show a mean value of six measurements and standard errors of the mean are depicted.

#### Root activity

The root activity was measured every second week during the course of the experiment. The sum of all the roots in one minirhizotron were calculated and every point in the diagram is a mean value of the six lysimeters in one experimental area (Figure 8).



**Figure 7.** Plant weights in *Salix viminalis* during year (1997) after harvest. Each point represents a mean value of six measurements.

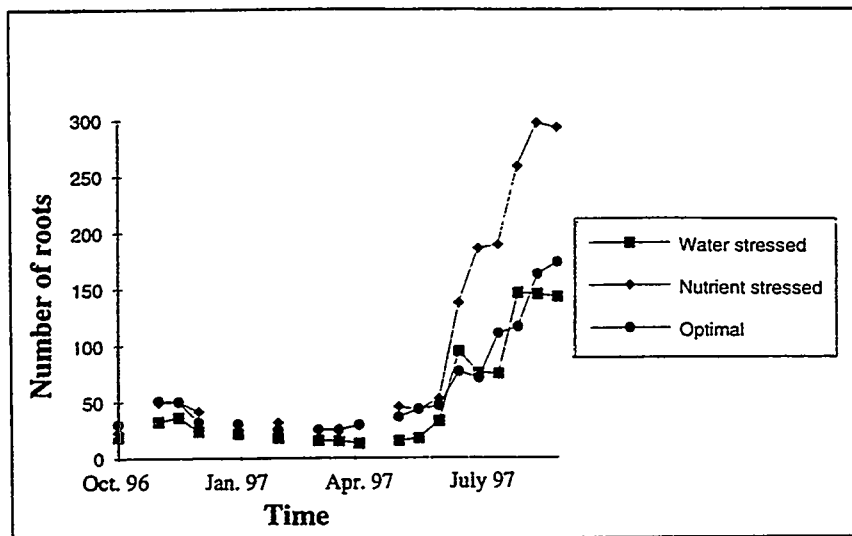


**Figure 8.** Root activity (number of roots/minirhizotron) in *Salix viminalis* during the first three years (1994-1996) of the experiment. To make the diagram easier to read smoothed curves were fitted to each of the treatments. Each point represents a mean value of six measurements.

To make the trends more clear the curves were smoothed by down weighting of the extreme values. This was done through a DWLS procedure (Distance Weighted Least Squares) in Systat V5.0 Package.

No obvious difference between the treatments was observed. The root activity varied during the growing season in the same way as the shoot growth. However, root activity never stopped completely. All plants, irrespective of treatment, had a rapid growth in May.

No particular treatment differences in root activity were observed in 1997, i.e. the year after harvest (Figure 9).



**Figure 9.** Root activity (number of roots/minirhizotron) in *Salix viminalis* in 1997, the year after the harvest. Each point represents a mean value of six measurements.

#### *Distribution of biomass*

Only the results from the harvests in June, July and August are discussed in this report. The results at harvest are presented in terms of the relative proportions of roots, stems and leaves. During 1994, when the treatments were identical, distribution of plant components in the different plots showed the same distribution pattern (Figure 10). During the second year a trend started; the plants cultivated under optimal conditions had a lower proportion of roots than the rest. This pattern is more obvious in 1996.

To examine if the distribution of biomass over the plant components differed between the treatments a mean value was calculated for the difference between the biomass fraction below ground for the different plants at every harvest. A standard error of the mean (SE) could then be calculated based on this value (Table 2). The table shows that the values differed significantly ( $P < 0.05$ ) between the plants which have been water stressed and those which have been cultivated under optimal conditions (column B) and between the plants which have been nutrient stressed and the unstressed plants (column C). The water stressed and the nutrient stressed plants (column A) only differed significantly year 1997.

All of the plants increased their share of biomass invested in the roots throughout the whole experiment. The plants cultivated under optimal conditions had the smallest increase and the water stressed plants had the highest (Figure 11).

The diagram was made by regression analysis. The share of the biomass invested below ground was calculated by the formula:

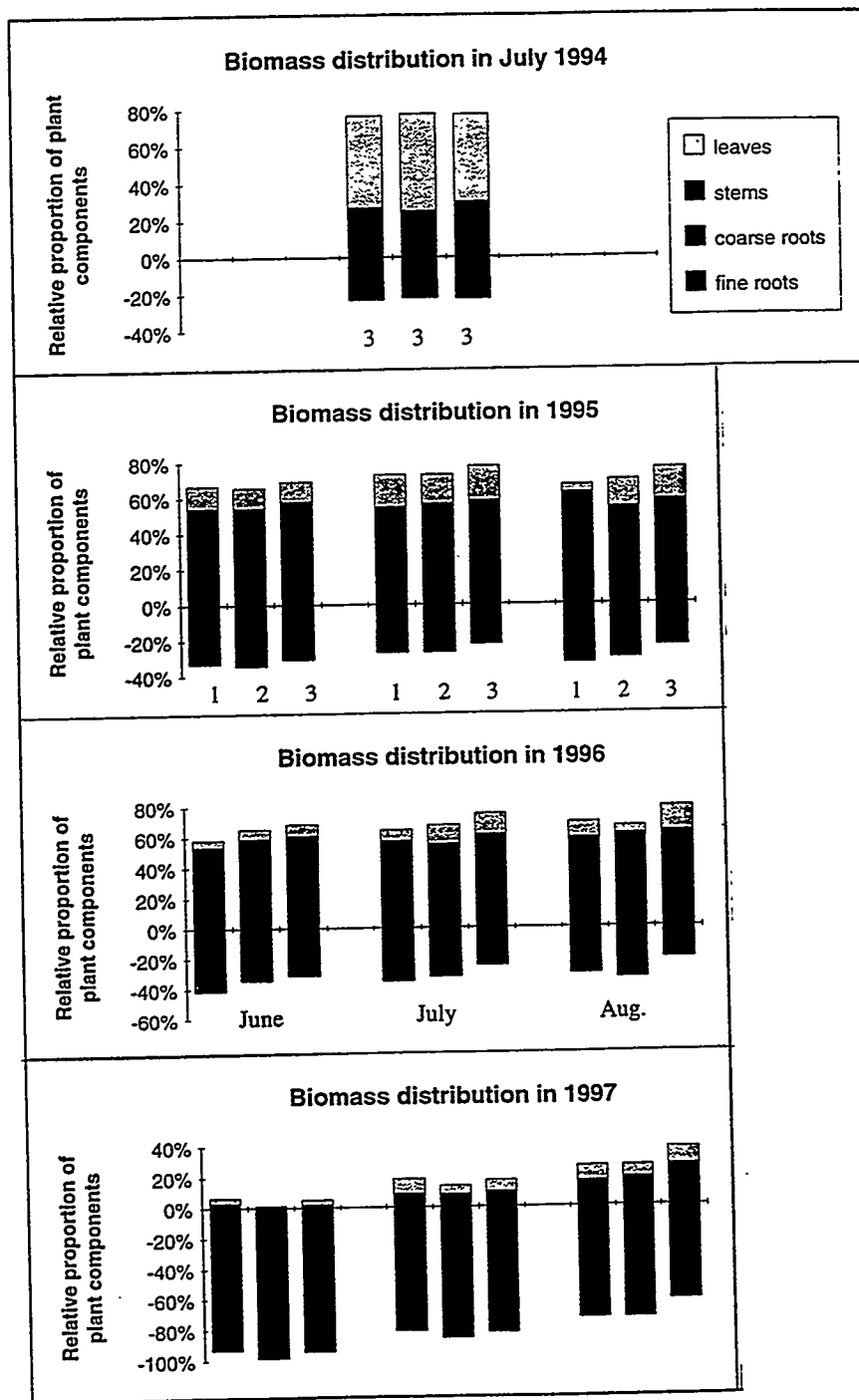
Biomass invested below ground as a percentage= $a+(b_1+b_2 \times \text{treatment}) \times \text{time}$ .

Where  $a$  is the intercept,  $b_1$  is an independent regression coefficient and  $b_2 \times \text{treatment}$  is a regression coefficient which depends on the area (i.e.  $b_2$  is multiplied with 1 in the line for the plants on plot one, with 2 for plants on plot two and with 3 for plants on plot three).

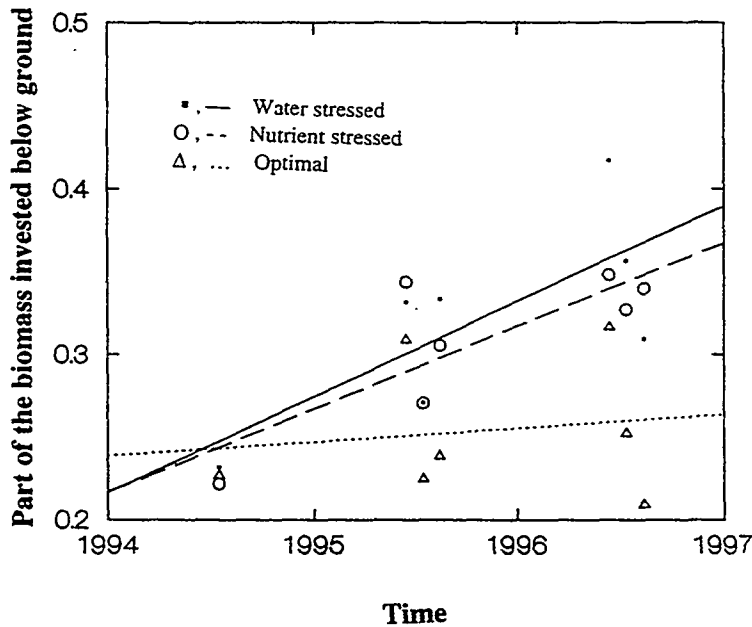
The value of  $b_1$  was calculated to 0.034957 and  $b_2$  to -0.000019 with an interval of confidence at 95% which did not include zero.

**Table 2.** *The differences in fraction of the biomass invested below ground were calculated. Plants from different plots harvested at the same occasion were compared. Mean values and standard errors of the mean were calculated for the differences in each year. Column A represents the difference between water and nutrient stressed plants, column B the differences between water stressed plants and plants cultivated under optimal conditions and column C differences between nutrient stressed plants and plants cultivated under optimal conditions.*

		A	B	C
Totally	Mean	-0.0006	0.0498	0.0504
	SE	0.0132	0.0169	0.0125
1995	Mean	0.0054	0.0544	0.0490
	SE	0.0117	0.0211	0.0093
1996	Mean	0.0228	0.0016	0.0788
	SE	0.0289	0.0012	0.0286
1997	Mean	-0.0500	-0.0123	0.0377
	SE	0.0025	0.0006	0.0019



**Figure 10.** Relative distribution of biomass in July 1994 and June, July and August in 1995 to 1997 in *Salix viminalis*. Bars number 1 represent plants grown under water limiting, bars number 2 represent plants grown under nutrient limiting and bars number 3 represent plants grown under non-limiting water and nutrient conditions. Each bar represents data from one plant.



**Figure 11.** Part of the total biomass invested below ground in *Salix viminalis*, as a function of plant age (one to three years) and water and nutrient availability. A linear model was fitted to each of the treatments.

## Discussion

### *The accomplishment of the experiment*

The aim of this experiment was to determine how clone 78 183 of *Salix viminalis* grow under water and nutrient stress.

It might be questioned if it is correct to draw conclusions about how the plants respond in situ from an experiment in which the plants have been cultivated in plastic containers filled with sand. The quartz sand makes it impossible for the water and nutrients to spread homogenous as the particles are big and the water holding capacity is low. Furthermore root growth might have been restricted physically by the walls of the lysimeter. In an earlier study with *Salix viminalis* Rytter (1997) studied how plants grow in lysimeters filled with sand compared to the growth in lysimeters filled with clay. She came to the conclusion that the plants cultivated in sand had a slower growth in the beginning, but after a while the growth was just as fast as for the plants cultivated in clay. This shows that the unusual growth substrate for *Salix viminalis* does not affect the growth in a longer perspective although the total production becomes a bit lower. All of her plants grew very well which indicates that the growth is not limited by the lysimeter. The experimental conditions should, however, be regarded as a compromise between studies under artificial conditions and cultivation in field since no root competition appears when the plants grow in lysimeters.

### *Shoot growth*

The plants cultured under optimal conditions had, as expected, the best growth. If the values for growth per plant are converted into growth per hectare the growth would have corresponded to 6 tons/hectare in 1995 and 12 tons/hectare in 1996. The higher growth in 1996 depended mainly on the age of the plants, but it might also have been affected by the weather conditions. The summer in 1995 was very hot and when the air is dry plants keep their stomata closed, even if the roots have access to water, and the production therefore is low.

The increase in dry weight in 1995 and 1996 for the plants which were nutrient stressed was 4 and 5 tons/hectare respectively and for the water stressed plants the corresponding rates were 3 and 2 tons/hectare respectively.

During the course of the experiment it was discovered that the water stressed plants also were nutrient stressed. The calculated optimal supply of nutrients was too small. The supply was increased thereafter but it is possible that the plants received too little nutrients in 1996 as well. This would explain the poor growth. A nutrient analysis of the leaves will give the answer to this question.

The growth period was shorter for the water stressed plants than for the nutrient stressed plants and the plants cultivated under optimal conditions. A probable reason is that when the plants are water stressed they close their stomata. If the water stress is really intensive or if it continues for a longer period of time the leaves stop to develop which results in a reduction in photosynthesis.

Values from year 1997 showed the same relationship between the plants from the different experimental areas i.e. plants cultivated under optimal conditions have the highest growth and the water stressed plants the lowest.

### *Root activity*

Different treatments did not affect the root activity in any obvious way. In all treatments the activity was lower during the winter but never stopped completely (Rytter 1997).

### *Distribution of biomass*

The distribution of biomass between plant components differed between plants from the different treatments. As expected the plants which received an optimal supply of water and nutrients had less root biomass at any time than those which were stressed (Ericsson 1981). They had 20-30% of their total standing biomass below ground compared to the stressed plants which had 30-40% of their total standing biomass below ground. The used values of root biomass as a percentage of the total biomass were measured at harvest. This is only a part of the total biomass invested in the roots since roots die off as the plant grows. Examination of the biomass distribution in a similar lysimeter-experiment has shown that the investment in roots, which at harvest seems to be 20% of the total biomass really amounts to 40% (Rytter 1996). If it is assumed that the real investment in roots is the double of the one measured at harvest the actual biomass placed in roots would be 40-60% of the total biomass for the unstressed plants and 60-80% for the stressed plants.

On the other hand leaves also are produced and decayed continuously and the biomass invested in leaves that is accounted for in this report is the one which is measured at

harvest. The above ground part is also larger than shown in this study but the difference ought to be much smaller than in the case with the roots.

All plants kept on increasing the ratio root biomass to total biomass through the course of the experiment. This is a bit surprising as the plants cultured under optimal conditions were expected to diminish their investment in roots from the second year and on. However they do not invest as much in roots as the stressed plants do.

#### *Future experiments*

To find out how efficient the plants use the water they are given, calculations of the water use efficiency (WUE), i.e. how many grams of biomass that are produced per litre of water, are required. The share of carbon in the form of  $^{13}\text{C}$  in the leaves would also be interesting to know as it gives a measurement of how much the stomata have been kept open and through that how much water that have been lost. It would also be interesting to repeat the experiment with other clones to see if the result would show any significant differences between the production, the period of production and the distribution of biomass.

#### **Conclusions**

The experiment shows that the growth of *Salix viminalis* is diminished when the plants are water or nutrient stressed. Water stress causes a closure of stomata as well as a loss of leaves while nutrient stress only causes loss of leaves. Consequently the uptake of carbon dioxide is prevented in two ways in the plants which get too little water. The water stressed plants in this experiment had a lower biomass production than the nutrient stressed, but if this is due to the reasons mentioned above or something else is not known as it has not been examined in this experiment.

What we do know is that the total production of biomass is lower in the stressed plants and more of the biomass is invested below ground at any time. Besides this the water stressed plants have a shorter growth period than the rest as they stopped growing one month earlier than the others.

#### **Acknowledgements**

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