

THE INTERACTIVE EFFECT OF WATER STRESS AND TEMPERATURE ON THE CO₂ RESPONSE OF PHOTOSYNTHESIS IN *SALIX*

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Seloste

VEDENVAJAUKSEN JA LÄMPÖTILAN YHTEISVAIKUTUS VESIPAJUN FOTOSYNTESISIN CO₂-VASTEeseen

Saapunut toimitukselle 7. 3. 1984

The interactive effect of water stress and temperature on the CO₂ response of photosynthesis is examined in the study on the basis of a restricted material. The measurements were carried out on a *Salix sp. cv. aquatica* plant using the closed IRGA system. The photosynthesis of the plant was followed as the CO₂ content of the air in the assimilation chamber fell from 720 ppm to the compensation point. A semi-empirical model, consisting of a non-rectangular hyperbola, was used to describe the CO₂ response of photosynthesis. The interactive effect of water stress and temperature was divided using the model into two components: the change in CO₂ conductance and the change in the photosynthetic capacity. The CO₂ conductance was not dependent on temperature when the willow plant was well watered but during water stress it decreased as the temperature increased. The photosynthetic capacity of the willow plant increased along with an increase in temperature when well watered, but during water stress temperature had quite the opposite effect.

INTRODUCTION

Water stress has been found to reduce photosynthesis by slowing down CO₂ diffusion. The mesophyll conductance is also reduced, as well as the stomatal conductance.

The photosynthetic capacity, i.e. photosynthesis at saturating CO₂ concentration and light intensity, has also been found to fall as a result of water stress (Mooney et al. 1977).

Hari & Luukkanen (1973, 1974) have shown that photosynthesis is especially reduced at high temperatures during water stress. Furthermore, Luukkanen (1978) found that the most important mechanism in this interactive effect is the reduction in the mesophyll conductance at high temperatures.

Nobel et al. (1978) have also reported that water stress and temperature have an interactive effect on photosynthesis.

Results which support those of Hari & Luukkanen (1973, 1974) and Luukkanen (1978) are presented in this preliminary report. In addition, the interactive effect is divided into two parts using a semi-empirical model of photosynthesis. First, the CO₂ conductance of the leaves becomes sensitive during drought to high temperatures, as has earlier been described (Luukkanen 1978, Nobel 1978). Secondly, the dependence of the photosynthetic capacity on temperature changes significantly during drought.

MATERIALS AND METHODS

Experimental material

Salix sp. cv. *aquatica* plants were grown from cuttings in 4 litre polystyrene pots containing fertilized horticultural peat (ST-400 Satoturve Ltd.). The plants were kept in a greenhouse where the day temperature was 20°C and night temperature 15°C. The length of the light period was 18 hours and that of the dark period was 6 hours. OSRAM HQI 400 W lamps, which had a quantum flux density of 350 $\mu\text{Em}^{-2}\text{s}^{-1}$ at the top of the shoots, were used as the light source. The plants were given sufficient water during the growing stage and were fertilized weekly with a multinutrient solution (Puutarhan Kastelunnoite, Kemira Ltd.) containing micronutrients (Kukkien Y-lannos, Kemira Ltd.).

One healthy plant was selected and transferred to the growth chamber for the measurements. The illumination and temperature conditions in the growth chamber were the same as those in the greenhouse. Water stress was induced gradually by stepwise reducing the amount of water given to the plant each day. The moisture content of the growing medium was followed by weighing the pot and plant each day before and after watering.

Measurement of CO₂ exchange

Net photosynthesis was measured using the closed IRGA system. About 20 of the uppermost leaves (about 500 cm² leaf area) were enclosed in the measuring cuvette which was kept at constant temperature by means of a water jacket. Two OSRAM HQI 400 W lamps were used as the light source, one of the lamps being placed above the cuvette and the other at the side. The quantum flux density from the lamp above the cuvette was 970 $\mu\text{Em}^{-2}\text{s}^{-1}$ in the upper part of the cuvette, and from the lamp at the side of the cuvette correspondingly 420 $\mu\text{Em}^{-2}\text{s}^{-1}$.

The measurement sequence was as follows: the CO₂ concentration of the air in the cuvette was initially raised to a level of about 1000 ppm by injecting CO₂-enriched air into the cuvette. The fall in the CO₂ concentration down to the compensation point was then

followed by means of a gas analyzer (URAS 2T). The reading of the gas analyzer and the temperature of the cuvette were recorded on punch tape at 100-second intervals using a data-logger (NOKIA Ltd.). The experimental set-up was approximately the same as that described by Luukkanen (1978). The volume of the measuring system was 6060 cm³ and the flow rate 1000 cm³ min⁻¹. Depending on the photosynthetic activity of the plant, it took between 20 and 120 minutes for the CO₂ concentration to fall from 1000 ppm to the compensation point. In cases where photosynthesis was very slow, the measurements were speeded up by periodically removing CO₂ from the system by means of a KOH solution.

The CO₂ concentrations and the corresponding values for net photosynthesis were calculated from CO₂ concentration in the middle of each measurement interval and the reduction in the CO₂ concentration during the measurement interval. When doing this it was assumed that the CO₂ concentration falls so slowly that the photosynthetic system remains in a steady state throughout the course of the measuring period. Only those measurements which were within the linear region (0 - 720 ppm) of the URAS have been used in the calculations.

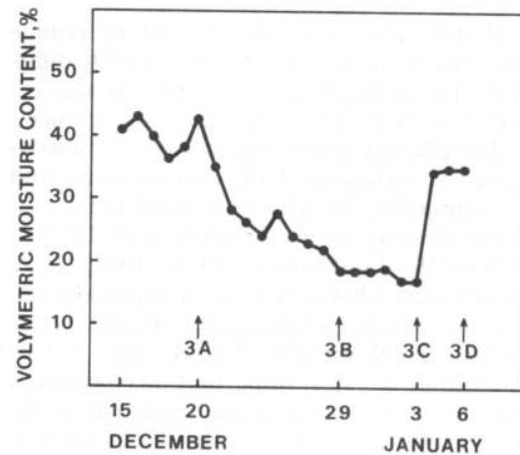


Figure 1. Development of soil moisture content (expressed as volume percentage) during the course of the experiment. The times when the results presented in Fig. 3 were obtained are indicated with arrows.

The CO₂ response of photosynthesis was measured once at four different temperatures (10, 16, 24 and 32°C) when the plant had been well watered (soil moisture 43 %), twice when the plant was subjected to water stress (soil moisture 18 and 17 %) and once again three days after rewatering (soil moisture 35 %). The development of the moisture content of the peat substrate during the measurement period 15. 12. 1977 - 6. 1. 1978 is depicted in Fig. 1.

The length and width of the leaves in the cuvette were measured to an accuracy of 1 mm after each series of measurements. The surface area of the leaves was calculated by multiplying the product of the width and the length by a constant, 0.68, which was determined separately from the same material.

Photosynthetic model

It is characteristic of the CO₂ response of net photosynthesis that it initially increases linearly, and then becomes saturated fairly rapidly. A large number of theoretical models (models derived from physiological theory, e.g. Gaastra 1959, Laisk 1970, Farquhar et al 1980), semi-empirical models (descriptive models where the parameters have a physiological interpretation, e.g. Thornley 1976) and empirical models (purely descriptive models, e.g. Enoch & Sacks 1978) have been presented for this response. Thornley's (1976) non-rectangular hyperbola, NRH, is a simple semi-empirical model which can be used to describe the CO₂ responses of net photosynthesis:

$$\begin{cases} \Theta P_G^2 - P_G(\beta C + P_{\max}) + \beta C P_{\max} = 0 \\ P_G = P_N + R \end{cases} \quad (1)$$

where P_N is the net photosynthesis, P_G the gross photosynthesis, C the CO₂ concentration, R , β , P_{\max} and Θ are the following parameters:

- β = the slope of the initial linear part of the model, conductance term
- R = the intercept term for the linear part of the model, the respiration term
- Θ = a parameter which determines the saturation rate. When $\Theta = 1$, the model is the Black-

man curve and when $\Theta = 0$, the model is the Michaelis-Menten curve.

P_{\max} = the saturation level, photosynthetic capacity.

The model initially increases linearly with respect to the CO₂ concentration, and it reaches saturation sufficiently rapidly. The saturation is not reached fast enough if the following Michaelis-Menten curve (rectangular hyperbola, RH) is used (Fig 2):

$$\begin{cases} P_G = \frac{\beta C P_{\max}}{P_{\max} + \beta C} \\ P_G = P_N + R \end{cases} \quad (2)$$

Marshal & Biscoe (1980) and Leverenz (1980) observed the same features as regards the light curves of photosynthesis. The parameter depicting the saturation level of RH is thus much larger than the real maximum value. For instance, the value of P_{\max} for RH in the case shown in Fig. 2 is 51 ng CO₂ cm⁻²s⁻¹, although the points in the figure indicate that the saturation level is reached at only half this value. Thus the value given by NRH for P_{\max} of 31 ng CO₂ cm⁻²s⁻¹ is much more realistic.

The main advantage of the non-rectangular hyperbola model in comparison to other semi-empirical and empirical models is the clear interpretation of the parameters. In addition, the estimates of the parameters are

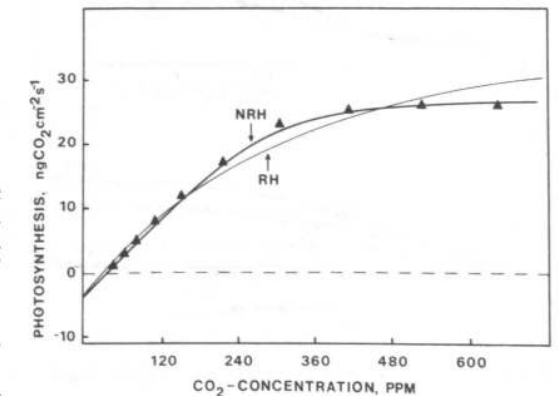


Figure 2. The measured CO₂ response ($T_a = 10^\circ\text{C}$, $W_s = 35\%$) and the NRH (Eq. 1) and RH (Eq. 2) fitted to the CO₂ response.

usually only slightly intercorrelated, which makes the estimation easier and improves the reliability of the estimates. The parameters have to be estimated iteratively, but the parameters which determine the linear part of the model, i.e. the initial slope (β) and the intercept (R), can initially be estimated using linear regression analysis. The maximum value observed for photosynthesis is a good initial estimate for the parameter P_{max} , and the

parameter (Θ) which determines the saturation rate can be preliminarily estimated by eye. Parameter Θ was found to vary so little in this study that it can be considered to remain constant in different experimental conditions ($\Theta = 0.95$). When photosynthesis is almost completely inhibited, the estimates for parameters β and R are inaccurate and, in addition, strongly intercorrelated.

RESULTS

As expected, both the saturation concentration and saturation level of photosynthesis increased as the temperature increased when the plant was watered normally (Fig. 3A). At the highest temperatures (24 and 32°C), the CO₂ curve saturated only slightly below 720

ppm. When the plant was subjected to drought, the saturation level fell as the temperature increased (Figs. 3B and 3C). The CO₂ responses after rewatering are very similar to what they were before drought conditions were imposed (Fig. 3D). The CO₂ com-

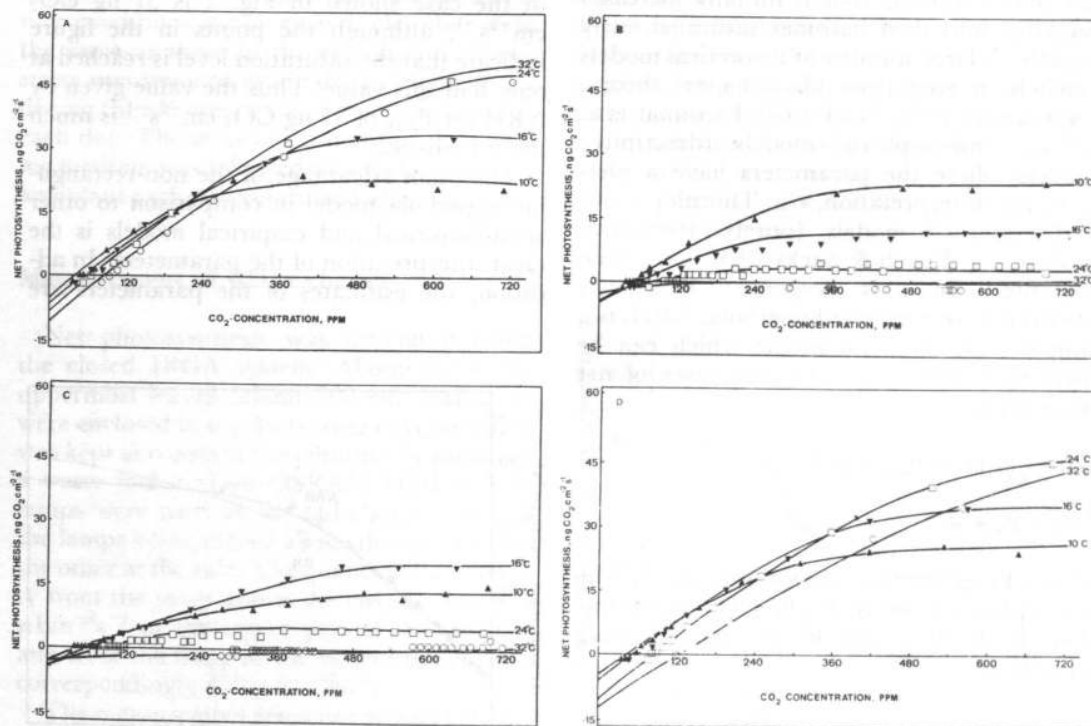


Figure 3. The measured CO₂ responses and the NRH: fitted to CO₂ responses at different temperatures.

- A: Well-watered, W_S = 43 %
- B: Water stressed, W_S = 18 %
- C: Water stressed, W_S = 17 %
- D: Rewatered, W_S = 35 %

penetration points are presented in Fig. 4 as a function of temperature.

The parameters of the CO₂ curves are presented in Fig. 5 as a function of temperature. The initial slope, β , which is considered to be the total CO₂ conductance, was almost completely independent of temperature when the plant was well watered. At low temperatures the values of fell slightly as a result of water stress, and especially strongly at high temperatures.

The intercept term, R , of the linear part of the model, which is considered to represent photorespiration (by the extrapolation method, e.g. Ludlow & Jarvis 1971), increased almost linearly with respect to temperature when the plant was well watered, being about 4 ng CO₂ cm⁻²s⁻¹ at a temperature of 10°C and about 10 ng CO₂ cm⁻²s⁻¹ at 32°C. When the plant was subjected to water stress, the values of parameter R were almost independent of temperature, ranging between 1 - 2 ng CO₂ cm⁻²s⁻¹.

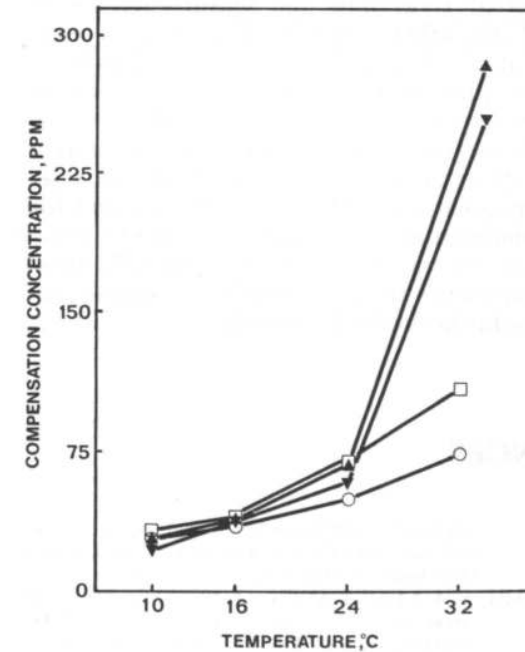


Figure 4. The observed CO₂ compensation points as a function of temperature.

- = Well-watered, W_S = 43 %
- ▲ = Water stressed, W_S = 18 %
- ▼ = Water stressed, W_S = 17 %
- = Rewatered, W_S = 35 %

The saturation level, P_{max} , which is considered to represent the photosynthetic capacity, increased when the plant received plenty of water from 30 ng CO₂ cm⁻²s⁻¹ to 60 ng CO₂ cm⁻²s⁻¹ as the temperature was increased from 10°C to 24°C. The value of P_{max} at a temperature of 32°C was only slightly greater than that at 24°C. Water stress had a similar effect on the saturation level as on the CO₂ conductance, since the value of P_{max} at 10°C is of the same order of magnitude as when the plant received plenty of water. However, when the temperature increased, P_{max} decreased very sharply and was already less than 10 ng CO₂ cm⁻²s⁻¹ at a temperature

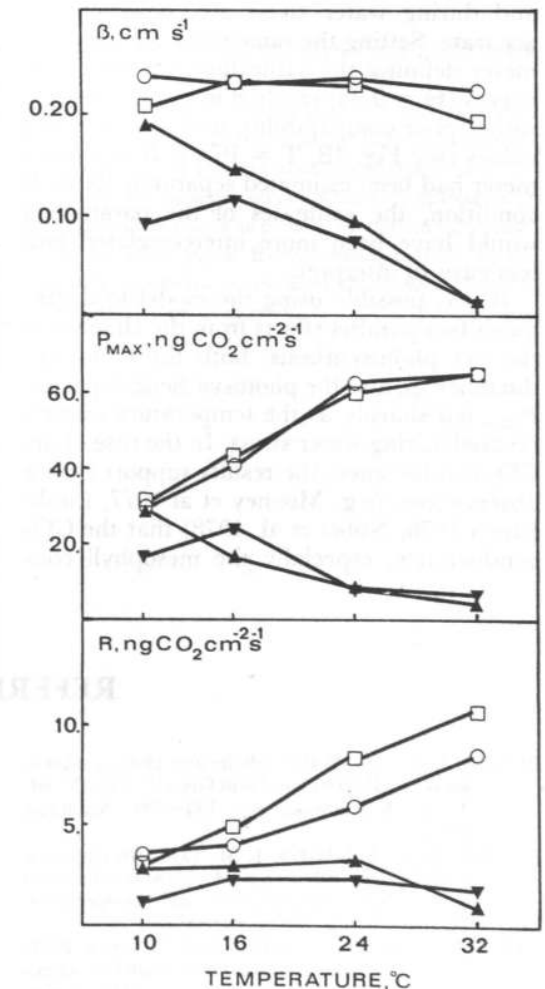


Figure 5. The parameters of NRH as a function of temperature. See Fig. 4 for key to symbols.

of 32°C. The temperature response of the photosynthetic capacity of the well-watered plant was thus an increasing function with respect to temperature and when the plant

was subjected to water stress the response was a decreasing function with respect to temperature.

DISCUSSION

The non-rectangular hyperbola model used here was well suited for depicting the observed CO₂ responses. However, this is partly due to serial autocorrelation of observations; the measurement procedure which was used automatically gives continuous CO₂ responses. The values of the parameters of the curves measured at high temperatures and during water stress are, however, not accurate. Setting the same value for the parameter defining the saturation rate in all the curves ($\Theta = 0.95$) resulted in some cases in a rather poor compatibility with the observed values (see Fig. 3B, $T = 16^\circ\text{C}$). If this parameter had been estimated separately for each condition, the estimates of the parameters would have been more intercorrelated and less easy to interpret.

It was possible using the model to distinguish two parallel effects from the changes in the net photosynthesis: both the CO₂ conductance, β , and the photosynthetic capacity, P_{max} , fell sharply as the temperature was increased during water stress. In the case of the CO₂ conductance, the results support earlier observations (e.g. Mooney et al 1977, Luukkanen 1978, Nobel et al. 1978) that the CO₂ conductance, especially the mesophyll con-

ductance, decreases during water stress especially at high temperatures. It is not possible to distinguish between stomatal conductance and mesophyll conductance because no simultaneous measurements of transpiration were available. A marked increase in the CO₂ compensation point at high temperatures during water stress, without any increase in photorespiration support results reported by Luukkanen (1978) for *Picea abies*.

Changes in the relationship between CO₂ conductance and its different components, and between photosynthesis and photorespiration, have been studied intensively in earlier investigations (e.g. Hsiao 1973, Boyer 1976), but no observations have been reported concerning the sensitization of the photosynthetic capacity, P_{max} , to high temperatures during water stress. A reduction in the photosynthetic capacity has, however, been reported (e.g. Mooney et al. 1977). As the measuring method used here was in some respects deficient, e.g. the steady-state assumption and lack of transpiration and leaf temperature observations, it would be advisable to test these results, especially those concerning the photosynthetic capacity, using further refined methods.

REFERENCES

- BOYER, J. S. 1976. Water deficits and photosynthesis. In *Water Deficits and Plant Growth*, Vol. IV (ed. T. T. Kozolowski) pp. 153–190. Academic Press.
- ENOCH, H. Z. & SACKS, J. M. 1978. An empirical model of CO₂ exchange of a C₃ plant in relation to light, CO₂ concentration and temperature. *Photosynthetica* 12 (2): 150–157.
- FARQUHAR, G. D., CAEMMERER, S. von & BERRY, J. A. 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. *Planta* 149: 78–90.
- GAASTRA, P. 1959. Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature, and stomatal diffusion resistance. *Meded. Landbouwhogeschool Wageningen* 59: 1–68.
- HARI, P. & LUUKKANEN, O. 1973. Effect of water stress, temperature, and light on photosynthesis in alder seedlings. *Physiol. Plant.* 29: 45–53.
- & LUUKKANEN, O. 1974. Field studies of photosynthesis as affected by water stress, temperature, and light in birch. *Physiol. Plant* 32: 97–102.
- HSIAO, T. C. 1973. Plant response to water stress. *Ann. Rev. Plant Physiol.* 24: 519–570.
- LAISK, A. 1970. A model of leaf photosynthesis and

photorespiration. In *Prediction and Measurement of Photosynthetic Productivity*. Proc. IBP/PP Technical Meeting, Trebon. pp. 295–306. Pudoc, Wageningen.

- LEVERENZ, J. 1980. Convexity and quantum efficiency of the light response curve of photosynthesis. In *Understanding and Predicting Tree Growth* (ed. S. Linder). Swedish Coniferous Forest Project. Techn. Rep. 25: 120–124.
- LUDLOW, M. M. & JARVIS, P. G. 1971. Methods for measuring photorespiration in leaves. In *Plant Photosynthetic Production: Manual of Methods* (Ed. Sestak, Z. & al.), pp. 294–315.
- LUUKKANEN, O. 1978. Investigations on factors affecting net photosynthesis in trees: gas exchange in clones *Picea abies* (L.) Karst. *Acta For. Fenn.* 162: 1–63.

MARSHAL, B. & BISCOE, P. V. 1980. A model for C₃ leaves the dependence of net photosynthesis on irradiance. I. Derivation. *J. Exp. Bot.* 31 (120): 29–39.

- MOONEY, H. A., BJÖRKMAN, O. & COLLATS, G. J. 1977. Photosynthetic acclimation to temperature and water stress in the desert shrub *Larrea divaricata*. *Carnegie Inst. Year Book* 76: 328–335.
- NOBEL, P. S., LONGSTRETH, D. J. & HARTSOCK, T. L. 1978. Effect of water stress on the temperature optima of net CO₂ exchange for two desert species. *Physiol. Plant.* 44: 97–101.
- THORNLEY, J. H. M. 1976. *Mathematical Models in Plant Physiology*. Academic Press.

Total of 15 references

SELOSTE

VEDENVAJAUKSEN JA LÄMPÖTILAN YHTEISVAIKUTUS VESIPAJUN FOTOSYNTTEESIN CO₂-VASTEESSEEN

Tutkimuksessa tarkastellaan suppean aineiston perusteella vedenvajauksen ja lämpötilan yhteisvaikutusta fotosynteesin CO₂-vasteen muotoon. Mittaukset tehtiin vesipajun taimesta suljetulla IRGA-systeemillä siten, että seurattiin yhteytyskammion ilman CO₂-pitoisuuden laskua 720 ppm:sta kompensatiopisteeseen. Fotosynteesin CO₂-vasteen kuvaamiseen käytettiin semiempiiristä mallia, nonrectangulaarista hyperbeliä. Mallin avulla veden-

vajauksen ja lämpötilan yhteisvaikutus voitiin jakaa kahteen komponenttiin: CO₂-konduktanssissa ja fotosynteesikapasiteetissa tapahtuviin muutoksiin. Hyvin kastellun pajun CO₂-konduktanssi oli lämpötilasta riippumaton ja vedenvajauksen aikana se pieneni lämpötilan kasvaessa. Hyvin kastellun pajun fotosynteesikapasiteetti kasvoi lämpötilan noustessa, mutta kuivuuden aikana lämpötilan vaikutus oli päinvastainen.