

Changes in photosynthetic capacity, carboxylation efficiency, and CO₂ compensation point associated with midday stomatal closure and midday depression of net CO₂ exchange of leaves of *Quercus suber**

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Abstract. The carbon-dioxide response of photosynthesis of leaves of *Quercus suber*, a sclerophyllous species of the European Mediterranean region, was studied as a function of time of day at the end of the summer dry season in the natural habitat. To examine the response experimentally, a "standard" time course for temperature and humidity, which resembled natural conditions, was imposed on the leaves, and the CO₂ pressure external to the leaves on subsequent days was varied. The particular temperature and humidity conditions chosen were those which elicited a strong stomatal closure at midday and the simultaneous depression of net CO₂ uptake. Midday depression of CO₂ uptake is the result of i) a decrease in CO₂-saturated photosynthetic capacity after light saturation is reached in the early morning, ii) a decrease in the initial slope of the CO₂ response curve (carboxylation efficiency), and iii) a substantial increase in the CO₂ compensation point caused by an increase in leaf temperature and a decrease in humidity. As a consequence of the changes in photosynthesis, the internal leaf CO₂ pressure remained essentially constant despite stomatal closure. The effects on capacity, slope, and compensation point were reversed by lowering the temperature and increasing the humidity in the afternoon. Constant internal CO₂ may aid in minimizing photo-

inhibition during stomatal closure at midday. The results are discussed in terms of possible temperature, humidity, and hormonal effects on photosynthesis.

Key words: Carboxylation efficiency – Compensation point (CO₂) – Photosynthesis (temperature, humidity) – *Quercus* – Sclerophyll.

Introduction

The midday depression of leaf CO₂ gas exchange in European Mediterranean sclerophylls was observed as early as 1933 in Brioni and Corsica by Guttenberg and Buhr (1935). A decrease in the net photosynthesis rate occurred when leaf temperatures increased above 30° C. While such a decrease might have been caused by direct temperature effects on photosynthesis, later studies demonstrated that changes in the net photosynthesis rate (NP) occur simultaneously with changes in stomatal conductance (Rouschal 1938; Hellmuth 1971; Schulze et al. 1974, 1975b; Eckhardt et al. 1975; Lange et al. 1975, 1982; Lange and Meyer 1979; Tenhunen et al. 1980, 1981). Closing the stomata may contribute to the decrease in photosynthesis rate by limiting the CO₂ supply. However, for leaves of apricot trees (*Prunus armeniaca*) growing in a desert climate at a run-off farm in Avdat, Negev, it was shown that the internal CO₂ partial pressure (P_i) of the leaf remains extremely constant during midday despite stomatal closure (Schulze et al. 1975b). This is remarkable, since the net photo-

* Dedicated to Professor Dr. Hubert Ziegler on the occasion of his 60th birthday

Abbreviations and symbols: CE=carboxylation efficiency; NP=net photosynthesis rate; PAR=photosynthetically active radiation; P_i=leaf internal CO₂ partial pressure; ΔW=water vapor mole fraction difference between leaf and air; Γ=CO₂ compensation pressure

tosynthesis rate can decrease, depending on the daily conditions and the degree of water stress, until compensation is reached. Thus, if calculations of leaf internal CO₂ pressure are reliable, strong changes in photosynthesis are indicated for leaves of species exhibiting midday closure in response to the midday increase in leaf temperature or to the decrease in humidity which usually occurs concurrently. The depression of the photosynthesis rate in ambient air begins at a relatively low temperature and an initial study of the daily time courses obtained with *Quercus suber* indicate that this occurs by 30° C and in some cases even at 27–28° C. Dunn (1975) reported an optimum temperature of approx. 25° C for net photosynthesis by leaves of sclerophyll shrubs in California and Chile. Schulze and Hall (1982) suggested a similarly low optimum temperature from time-course data obtained with *Artemisia herba-alba*, which exhibits midday stomatal closure.

Regulation of gas exchange during midday, on days when stomatal closure occurs and depression of CO₂ exchange is exhibited, is interesting because of the possible selective advantage in water-use efficiency conferred by this mechanism on species experiencing long-term drought stress. Stocker (1956) and, later, others (Mooney and Dunn 1970; Schulze et al. 1975a; Hall et al. 1976) called attention to the amount of water saved by the plant, when strong closure of stomata occurs at midday. Theoretical analyses have suggested that under certain hot and dry weather conditions a leaf may fix the maximum possible quantity of CO₂ for the use of a set but limited amount of water by decreasing conductance at midday and by restricting high rates of CO₂ uptake and transpiration to the early morning and late afternoon (Cowan and Farquhar 1977; Cowan 1982). Moderately stressed sclerophylls are, during the dry season, in a natural situation in which the optimization of CO₂ exchange might be useful. A detailed study of the NP versus P_i function during midday depression would help define the response of NP to light intensity, temperature, and P_i, and an accurate description of the interactive effects of light, temperature, humidity, and P_i on net photosynthesis during midday is required in order to evaluate critically the optimization theory.

If stomata respond to humidity (Schulze et al. 1974) in a feed-forward fashion (Farquhar 1978) and close at midday, we must explain why, despite a constant P_i and an increasing temperature which might be expected to increase photosynthetic capacity, the net photosynthesis rate decreases. (By photosynthetic capacity, we mean the CO₂-satu-

rated rate of net photosynthesis for particular light and temperature conditions). One possibility is that photorespiration is strongly activated, thereby reducing the ambient net photosynthesis rate and releasing CO₂ which contributes to maintenance of constant P_i; the leaf may then be protected from photoinhibition as hypothesized by Osmond and Björkman (1972). Leaf internal CO₂ partial pressures below 100 μbar may lead to the inhibition of photosynthetic processes of leaves of sclerophyll shrubs at high light intensity and high temperature (Mahall and Schlesinger 1982). Alternatively, the photosynthesis rate at high temperatures may be reduced by a mechanism other than photorespiration and stomata might respond in order to maintain a constant ratio of leaf internal CO₂ partial pressure to leaf surface partial pressure (Ball and Berry 1982). Finally, photosynthesis rate and conductance may be determined independently during the midday depression and in response to either the same or different plant internal factors. This possibility is made plausible by the observation that the stress hormone, abscisic acid, which strongly closes stomata (Raschke 1975), can also depress photosynthesis in certain cases (Raschke 1982).

The strong changes observed in ambient-air net photosynthesis rates during the midday depression may further indicate the following. If P_i remains constant, then the carboxylation efficiency (CE) and-or the CO₂ compensation pressure (*I*) must be shifted strongly. The carboxylation efficiency should decrease and the compensation point might be expected to increase as the leaf experiences a higher temperature and lower humidity. Previous studies restricted to crop plants have indicated that an increase in temperature from 25° C to 35° C does not lead to a appreciable change in CE (Badger and Collatz 1977; Ku and Edwards 1977; Peisker et al. 1979; von Caemmerer and Farquhar 1981; Weis 1981; Farquhar and von Caemmerer 1982).

Photosynthetic capacity at CO₂ saturation (P_M) may or may not be decreased at midday. If the capacity is decreased beginning at temperatures between 27 and 30° C, then such an unusual result could not be explained by presently known mechanisms. If capacity remains high, then a depression of photosynthesis at midday would indicate that, as the temperature increases and the humidity decreases, the leaf operates at ever lower points on the NP versus P_i response curve. Thus, in contrast to other situations which have been reported (von Caemmerer and Farquhar 1981; Ball and Farquhar 1984a, 1984b), the operation point at midday

would be relatively low on the linear portion of the response curve.

The studies described here were undertaken to clarify arguments related to the regulation of gas exchange of sclerophyll leaves which exhibit midday depression. In particular, we have attempted to describe in detail and from observations in the natural habitat, changes in the NP versus P_i response curve that take place in *Q. suber* leaves during days on which midday closure and depression of gas exchange occur. We have restricted our discussion to results obtained with either non-water-stressed or moderately water-stressed plants, since in these experiments transpiration rates and conductance values, even at midday, are relatively high and calculated P_i values are most reliable. In our analysis, we have considered the pattern in daily change that is found for Γ , CE, and P_M.

Material and methods

The experiments were conducted during a warm-weather period after the first autumn rains in the fall of 1982, in a natural macchia of a type related to the *Quercetalia ilicis* as described by Braun-Blanquet (1952; see Tenhunen et al. 1980) near Lisbon, Portugal. The time period was chosen because release of the plants from extreme stress conditions opened stomata and increased photosynthetic rates. Thus, errors in estimating stomatal conductance and internal leaf CO₂ partial pressure were reduced. In addition, the plants were still experiencing relatively high temperatures naturally (daily air temperature maxima between 25 and 35°C) and could be expected to respond in a natural way to the "standard" temperature time course (described below) imposed during experimentation. The shrubs of *Quercus suber* chosen were approx. 2 m in height and had crowns approx. 1 m in diameter.

Branch ends with several leaves were enclosed in gas-exchange cuvettes (Walz Meß- und Regeltechnik, Effeltrich, FRG) which were part of a mobile laboratory. The methods used for measurement of gas exchange were essentially the same as described by Schulze and Küppers (1979). The system could be controlled to either track outside air temperature and/or humidity or set values for these factors could be imposed. The Peltier heat exchangers and the air-conditioning parts of the cuvette were plated with nickel to avoid water exchange from the materials. Plexiglas parts of the cuvette were coated with self-adhesive teflon film (Type S-115, du Pont; Newtown, Conn., USA). Experiments conducted with empty cuvettes indicated a rate of transpiration of zero during entire "standard" day time courses. Dew point was set at approx. 10°C and in accordance with prevailing atmospheric conditions. Any increase in humidity from transpiration was compensated for, with a variable rate of pumping of air through a closed loop bypass with a cold trap maintained at a temperature approximately one degree lower than the dew point of the incoming gas. Metal-jacketed teflon tubing was used to conduct the gases from the incoming dew-point cold trap until the outgoing dew-point mirror of the chamber and in the entire bypass (see Schulze and Küppers 1979). Measurement of dew points and transpiration rate was discussed by Tenhunen et al. (1981).

The absolute CO₂ partial-pressure of air entering the measurement cuvettes and the CO₂ partial-pressure difference

across the measurement cuvettes were measured with BINOS infra-red gas analyzers (Leybold-Heraeus, Hanau, FRG). Gas mixtures with varying CO₂ partial pressures in air were obtained by adding pure CO₂ to CO₂-free air. Flows of each were determined with mass-flow controllers calibrated with volume-metering devices. Partial pressures of CO₂ between 0 and 3000 µbar external to the leaf were used to establish the carbon-dioxide dependency of net photosynthesis. Photosynthesis rates were calculated from CO₂ partial-pressure differences across the cuvette and the flow rate of air through the cuvette. Corrections were made for water vapor condensation from the measurement gas stream in a cold trap before the CO₂ analyzer according to the equations of von Caemmerer and Farquhar (1981). In those cases where twigs were necessarily included in the cuvette, net photosynthesis rates of the leaves were obtained by correction for the temperature-dependent twig respiration determined after removal of the leaves. Through careful selection of material, this correction was always very small (less than 5% of net photosynthesis rate). All rates are expressed on a projected leaf-area basis. To enable conversion to a dry-weight or chlorophyll basis, the average dry weight per unit area was determined as 0.016 g cm⁻² and the average chlorophyll content per unit area as 0.051 mg cm⁻².

Leaf temperatures were measured with copper-constantan thermocouples. Leaf conductance was calculated from leaf transpiration rate and from the water vapor mole-fraction difference between leaf and air (ΔW) assuming water saturation at leaf temperature in the leaf intercellular air spaces. Leaf internal CO₂ pressure was estimated from leaf conductance and net photosynthesis rates using the equations of von Caemmerer and Farquhar (1981) and as discussed by Sharkey et al. (1982). Incident photosynthetically active radiation (PAR) was measured in the cuvettes with quantum sensors from LiCor, Inc. (Lincoln, Neb., USA). Leaf material was illuminated naturally or experiments were conducted at constant saturating light intensity (described in figure captions). Constant light was provided with nine quartz-halide projector lamps (EYF 75 W; General Electric, Frankfurt a. M., FRG). The temperature was either held constant or was controlled to produce comparable conditions on consecutive days according to a "standard" time course modelled after measured microclimate conditions (see Tenhunen et al. 1980, 1981). Since the dew point of the incoming gas stream was constant, ΔW varied simultaneously with and in a similar pattern to temperature (illustrated in the figures). To determine the change in leaf gas-exchange characteristics with time, different CO₂ partial pressures in air were supplied to the leaves on consecutive days. Leaf water potentials were measured with a pressure chamber (Scholander et al. 1965).

Results

The "standard" temperature and humidity conditions imposed during daily time-course experiments and the leaf response of gas exchange and stomatal conductance to changes in light, temperature, and ΔW , with 350 µbar CO₂ in the air external to the leaf, are shown in Fig. 1. The conditions closely resemble those occurring naturally in September at the study site, and the behavior in gas exchange closely reflects natural behavior observed on other days when actual air temperature and humidity was followed in tracking mode. Incident light intensity varied naturally; air temperature

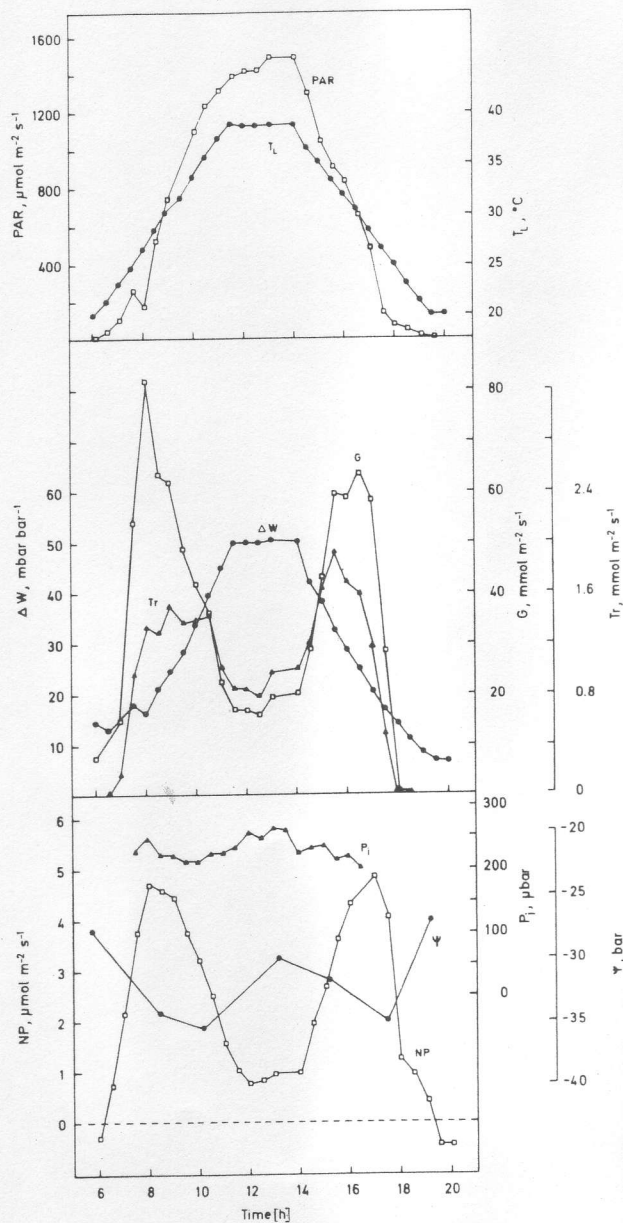


Fig. 1. A "standard" daily time course of gas exchange of *Quercus suber* leaves observed on September 5, 1982. Shown are the naturally occurring time course for photosynthetically active radiation flux (PAR) incident on leaves in the gas-exchange cuvette, controlled time course for leaf temperature (T_L) and water vapor mole-fraction difference between leaf air spaces and external chamber air (ΔW), total leaf conductance for water vapor (G), leaf transpiration rate (Tr), leaf net photosynthesis rate (NP), leaf xylem water potential (ψ), and internal leaf CO₂ pressure (P_i)

and leaf temperature were increased in the morning by approx. $3^\circ \text{C} \cdot \text{h}^{-1}$. A constant temperature (leaf temperature approx. 38°C) was maintained for 3 h around midday when the light intensity was maximal. During the afternoon, the temperature was decreased in steps equal to those used during the

morning period. The decrease in humidity at midday (increase in ΔW) was directly dependent on changes in leaf temperature; a maximum ΔW of approx. 50 mbar bar^{-1} was reached at noon. The pattern of temperature and humidity changes was chosen to produce a similar combination of environmental variables in the morning and in the afternoon. Thus, the responses during these two time periods can be compared and daily time influences on response may be scrutinized.

When exposed to the "standard" time course of environmental variables, transpiration rate, photosynthesis rate, and leaf conductance reached maximum values early in the morning and late in the afternoon. Only slight differences in the peak values are seen on this day, although conductance was higher in the morning and transpiration was higher in the afternoon. Air temperature measured at other locations in the canopy reached a maximum of approx. 35°C at 1:00 p.m. Stomatal closure throughout the plant canopy apparently resulted in strong recovery in leaf water potentials at this time. Leaf water potentials became more negative again late in the afternoon when temperatures in general decreased. Stomatal re-opening and stimulation of net photosynthesis occurred immediately in the case of the experimental leaves with a decrease in temperature at 2:00 p.m. The leaf internal CO₂ partial pressure remained quite constant despite in 18°C change in leaf temperature. Only at the highest temperatures at midday did P_i increase slightly. This is probably explained indirectly by the decrease in net photosynthesis rate, which leads to a smaller CO₂ differential across the gas-exchange chamber and slightly higher average CO₂ pressure external to the leaf in the cuvette air. The transpiration/NP ratio increased by a factor of approximately three between early morning and midday.

In order to clarify changes in the CO₂ response of *Q. suber* leaves occurring during the day, experiments were conducted in which three different CO₂ pressures were provided in the incoming gas stream on three consecutive days during which light and leaf temperature were essentially identical (as in Fig. 1). The results of such an experiment are shown in Fig. 2. With $350 \mu\text{bar}$ CO₂, the time course shows the natural behavior of the leaves. By decreasing the external CO₂ partial pressure to $105 \mu\text{bar}$, information was obtained which, combined with the $350\text{-}\mu\text{bar}$ response (see below), allowed determination of the initial slope of the CO₂ response curve; by increasing the external CO₂ partial pressure to $2000 \mu\text{bar}$, the time course of photosynthetic capacity at CO₂ saturation was

observed. With an incoming CO₂ pressure of 2000 μbar , P_i was approximately constant at 1100 μbar (not shown). Leaf internal CO₂ partial pressure obtained with 350 μbar and 105 μbar CO₂ external to the leaf is indicated as a function of time of day in Fig. 2.

To ensure that longer-term treatment with high CO₂ was not responsible for the decrease in the photosynthetic capacity observed at midday, the leaves were subjected to an immediate temperature step when the light was turned on in the morning (Fig. 3). Thereafter, constant conditions were maintained. The external CO₂ partial pressure was 2500 μbar and the light intensity was saturating (1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR). With the change from 19° C to 29° C (attained at 7:00 a.m.), the net photosynthesis rate increased rapidly to approx. 23 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and then remained constant at least until 12:30 p.m. (which on a "standard" day would be the middle of the period with constant maximum leaf temperature). With a temperature step to 37° C (attained at 7:40 a.m.), the net photosynthesis rate initially increased rapidly to approx. 12.0 $\mu\text{mol m}^{-2} \text{s}^{-1}$; at this point, the leaf temperature was approx. 34° C. Then the stomata closed and NP decreased sharply despite increasing P_i to a minimum at 8:00 a.m. after which NP increased (simultaneously with renewed stomatal opening) to a stable value of approx. 15.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ which again was maintained into the midday period. A decrease in CO₂ pressure external to the leaf to 2000 μbar (P_i changing from 1350 μbar to 1080 μbar) decreased net photosynthesis only slightly (arrow in Fig. 3). The "standard" day-temperature time-course data obtained with the same leaves at 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR and 2500 μbar external CO₂ provided a peak value for CO₂ uptake at 29° C in the morning of 24 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and a value at midday during constant conditions (37° C) of 16 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Thus, the values obtained in time courses of the type described, even at high CO₂ pressure, seem to be close to steady-state values and the accumulation of photosynthates does not cause the large decrease observed in photosynthetic capacity at midday. The absolute rates are higher than shown in Fig. 2 because of greater recovery in plant water potential. Day-to-day changes in water status cannot be eliminated from these experiments under natural conditions.

While a decrease in leaf temperature in the afternoon resulted in an increase in photosynthetic capacity in our experiments, the afternoon peak is often not as high as the morning peak (Fig. 2). In other words, in some experiments overall photo-

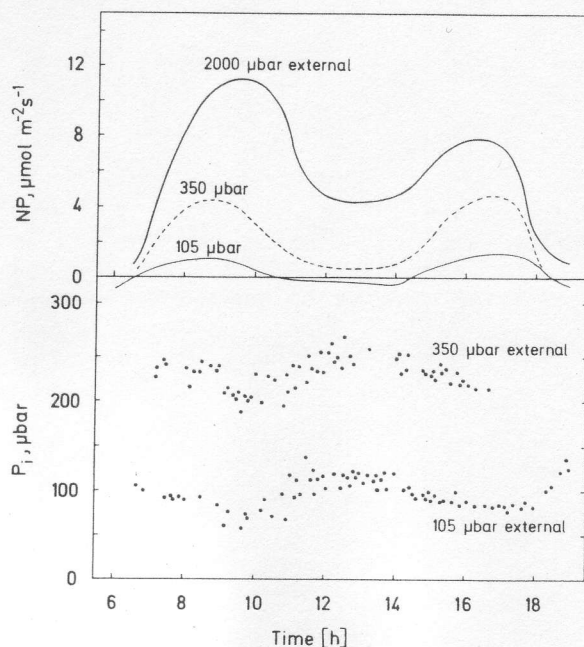


Fig. 2. Time course of rate of net CO₂ uptake of *Quercus suber* leaves exposed to "standard" day conditions of PAR, leaf temperature, and ΔW as shown in Fig. 1 and supplied with either 2000 μbar , 350 μbar , or 105 μbar CO₂ in the incoming air stream to the cuvette. Lower portion of the figure shows calculated leaf P_i at different times with 350 μbar and 105 μbar supplied in the incoming air stream

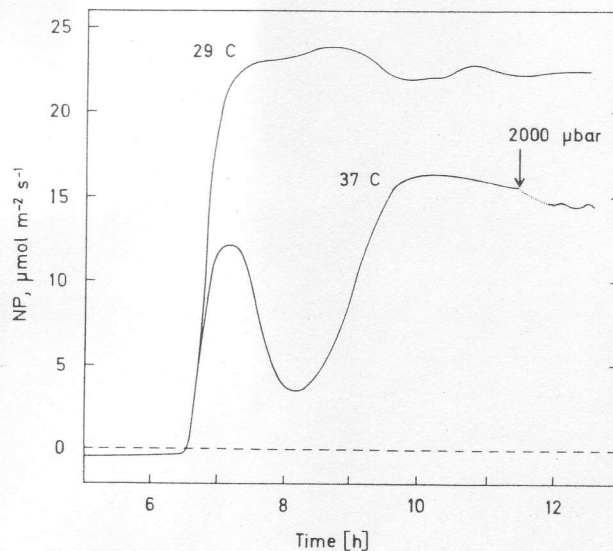


Fig. 3. Time course of net CO₂ uptake of *Quercus suber* leaves with increase in light intensity (PAR) from zero to 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at 6:30 A.M. and rapid increase in leaf temperature from 19° C to 29° C (attained at 7:00 a.m.) after which leaf temperature was held constant; and similarly with rapid increase in leaf temperature to 37° C (attained at 7:40 a.m.). Pressure of CO₂ in air supplied to the leaves was 2500 μbar . Arrow indicates change in the CO₂ pressure supplied to the leaves to 2000 μbar

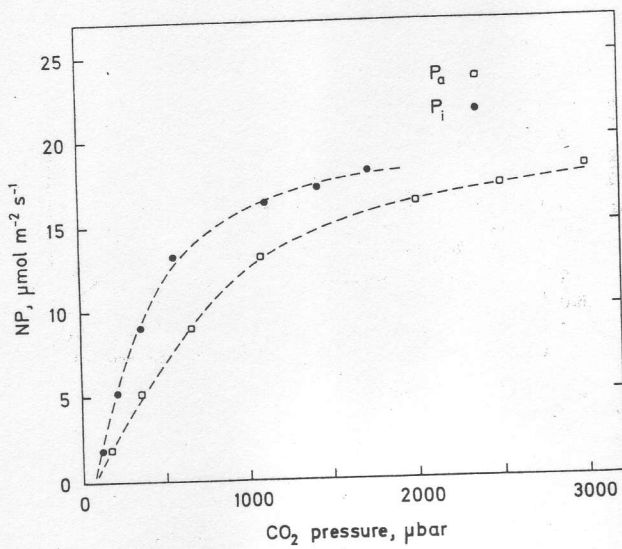


Fig. 4. Dependency of net photosynthesis rate on external air CO₂ pressure (P_a) and on internal air-space CO₂ pressure (P_i) under midday conditions. Leaf temperature 37° C, ΔW approx. 50 mbar bar⁻¹, and light intensity 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR

synthetic capacity showed a tendency to decline slightly in the afternoon. This response is not always predictable and could still involve long-term changes in the ability to remove photosynthetic products from the leaves or might result from the longer-term effects of midday stress conditions.

To demonstrate conclusively that CO₂ saturation is achieved at midday with the external CO₂ pressure between 2000 and 2500 μbar and despite strong stomatal closure, the dependency of net photosynthesis rate on external air CO₂ pressure and on internal leaf air-space CO₂ pressure was determined as shown in Fig. 4 for midday conditions (37° C and 1600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR). At 2000 μbar external CO₂, an internal CO₂ pressure of approx. 1200 μbar is obtained and net photosynthesis is very close to CO₂ saturation. It is interesting that the rate of net photosynthesis at normal atmospheric air CO₂ partial pressure (P_{350} – a normal air partial pressure for CO₂ of 350 μbar was determined in Sobreda) is only 27% of the maximum rate and that NP continues to increase with increase in P_i between 500 and 1000 μbar . The operating point with respect to maximum, estimated as $P_{350}/P_{\text{saturation}}$ from data like those in Fig. 2, is shown as a function of time of day for two experimental series in Fig. 5. Similar low values of $P_{350}/P_{\text{saturation}}$ indicate again that a relatively high photosynthetic capacity is present and the operating point is situated low on the linear portion of the NP versus P_i response curve.

An estimate of the initial slope of the CO₂ re-

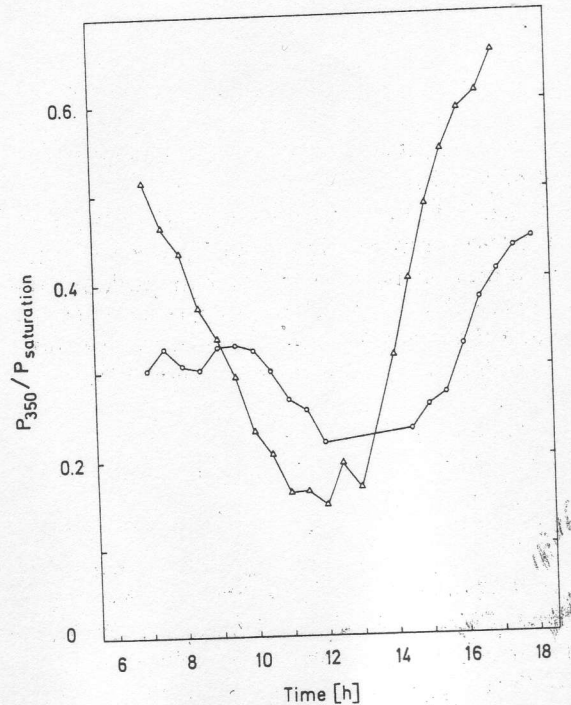


Fig. 5. Time course under "standard" day conditions of PAR, leaf temperature, and ΔW as shown in Fig. 1, for the ratio of net photosynthesis rate with ambient gas concentration to the net photosynthesis rate at CO₂ saturation ($P_{350}/P_{\text{saturation}}$). Data are shown for two experimental series; circles correspond to plants with water status as shown in Fig. 1; triangles correspond to plants which have more fully recovered from water stress (pre-dawn water potential of -5 bar)

sponse curve at different times of the day, CE, was obtained from the "standard" daily time courses obtained with 350 μbar and 105 μbar CO₂ external to the leaf. The average rates of CO₂ uptake over half-hour intervals were plotted versus average internal air-space CO₂ pressure as shown in Fig. 6. The estimated slope of the CO₂ response curve decreased as temperature increased and humidity decreased during the morning and increased again as temperature decreased and humidity increased in the afternoon. Little change occurred during midday when temperature and ΔW were constant. An estimate of the CO₂ compensation point, Γ , was also obtained from the data shown in Fig. 6 by extrapolation. While a certain inaccuracy may be expected as a consequence of determining Γ from only two measurement points, the shift with time of day is qualitatively correct because with 105 μbar external CO₂, net CO₂ uptake occurs during the morning and late afternoon, while CO₂ evolution is observed at midday. The carboxylation efficiency and CO₂ compensation point are related to measured leaf temperature and to ΔW in Fig. 7.

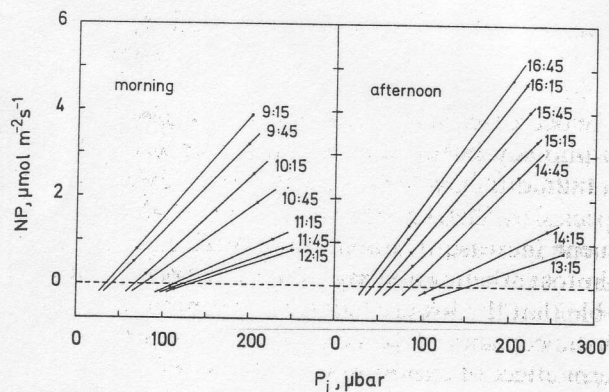


Fig. 6. Initial slope of the CO₂ response curve (carboxylation efficiency - CE) obtained at different times of the day with "standard" day conditions of PAR, leaf temperature, and ΔW as shown in Fig. 1. Data correspond to those shown in Fig. 2.

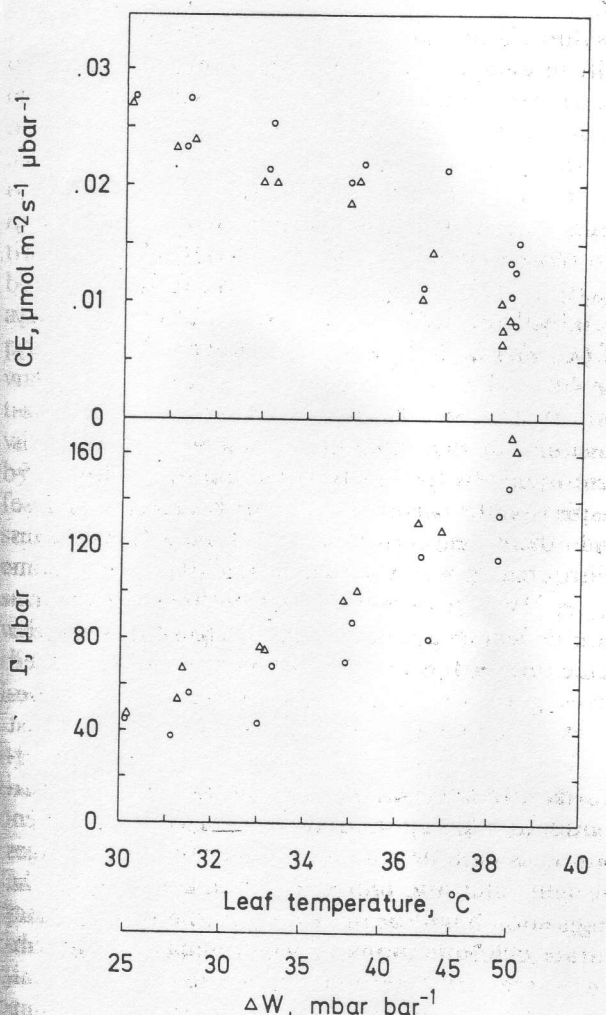


Fig. 7. Initial slope of the CO₂ response curve (carboxylation efficiency - CE) and extrapolated CO₂ compensation pressure (Γ) obtained at different times during a "standard" day plotted against leaf temperature and water vapor mole-fraction difference between leaf and air (ΔW). Data from two experimental series

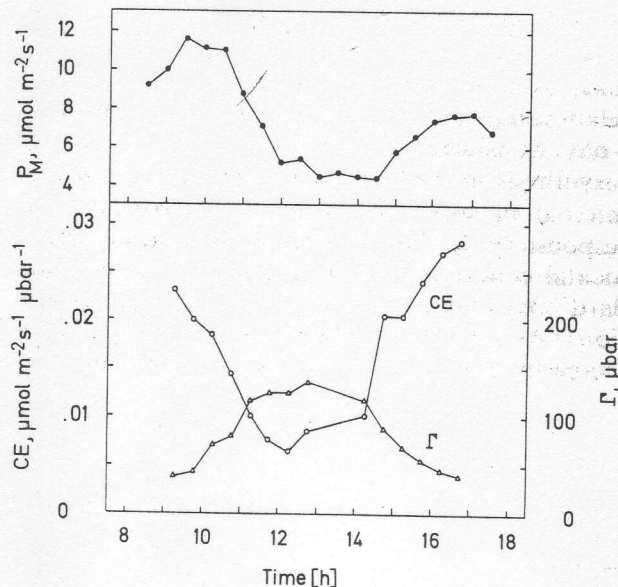


Fig. 8. Daily time course found for leaf photosynthetic capacity (P_M) measured at 2000 μbar external CO₂ pressure, for carboxylation efficiency (CE), and for leaf CO₂ compensation point (Γ) on a "standard" day as shown in Fig. 1

The change in photosynthetic capacity at CO₂ saturation, CE, and Γ on a "standard" day is depicted in Fig. 8. Photosynthesis at midday decreases and the carbon-dioxide partial pressure of the internal air space remains at a relatively constant level (Fig. 1) as a result of the depression of photosynthetic capacity, the decrease in carboxylation efficiency, and the increase in the compensation point. Carboxylation efficiency and compensation point begin to change before photosynthetic capacity is reduced. On "standard" days, the increase in natural light intensity increases photosynthetic capacity until light saturation is reached at approx. 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR. Thus, light saturation is reached between 9:00 and 10:00 a.m., just when the maximum in photosynthetic capacity is observed during the daily time course. Thereafter, temperature and humidity changes depress photosynthetic capacity.

Discussion

The decrease in leaf conductance and concurrently in the rate of leaf net photosynthesis during midday depression of gas exchange is a complex, regulated response of leaf metabolism to the imposition of "short-term" stress conditions. The results described here obtained with leaves of *Q. suber* indicate that the gradual midday stomatal closure is accompanied by a continuous decrease in photosynthetic activity. Except when an extremely rapid

increase in temperature and decrease in humidity may transiently disrupt photosynthetic CO₂ uptake, as seen in Fig. 3, approximate steady-state relationships are maintained during the course of a day. Considered in terms of the response of photosynthesis to the CO₂ partial pressure of the leaf internal air space, the basic characteristics of the response curve change continuously with environmental conditions during the course of a "standard" day typical for mediterranean climate conditions. At saturating light intensity with *Q. suber*, increasing leaf temperature and the consequent increasing ΔW result in a decrease in photosynthetic capacity (the CO₂-saturated rate of net photosynthesis was measured here with 2000 μ bar CO₂ external to the leaf), a decrease in the carboxylation efficiency (initial slope of the CO₂ response), and an increase in leaf CO₂ compensation point. As a result of these changes, the leaf internal CO₂ pressure remains constant or increases only slightly despite stomatal closure. Thus, the observations agree with the hypothesis of Osmond and Björkman (1972; Osmond et al. 1980) that stomatal closure during periods of high temperature and high light intensity should be accompanied by metabolic changes which maintain high internal CO₂ pressure and thereby hinder photoinhibition (Powles and Critchley 1980). The role played by photorespiration in changing the shape of the photosynthetic CO₂ response curve must still be investigated. Since water stress has been shown to increase susceptibility to photoinhibition (Björkman et al. 1981), it may be that the processes which change to produce these observed shifts in carboxylation characteristics, change even more strongly in leaves of *Q. suber* and perhaps in other mediterranean sclerophylls as water stress increases and as stomata close tightly for longer periods of the day during the summer dry period (Tenhunen et al. 1982).

If the photosynthetic capacity observed in these experiments is considered only as a function of temperature, an optimum temperature is found between 30 and 35°C. This in itself is not remarkable, although in other cases where photosynthetic capacities have been measured for plants adapted to high temperature and high light conditions, higher optimum temperatures have been found (Björkman et al. 1978; Mooney et al. 1978). The degree to which photosynthetic capacity suddenly decreases at high temperature is striking. At 37°C the capacity is only 65% of that at 29°C (Fig. 3). Ball and Farquhar (1984a, b) have reported changes in the photosynthetic capacities of leaves of mangrove species exposed to salinity stress condi-

tions. In contrast, the changes in capacity observed in *Q. suber* leaves occur over a much shorter time period and are rapidly reversed when the temperature decreases and the humidity increases. Changes in photosynthetic capacity in response to changes in humidity have been reported recently for several species by Sharkey (1984). In that study, a subsequent increase in humidity allowed a recovery in photosynthetic capacity of approx. 50%. It is possible that the lowered afternoon peak in photosynthetic capacity of *Q. suber* is the result of a longer-term effect of exposure to conditions of high evaporation.

On a day with a "standard" temperature time course and at light saturation, changes in the carboxylation efficiency and the compensation point parallel changes in the photosynthetic capacity of *Q. suber* leaves and determine the rate of net photosynthesis at ambient CO₂ pressure (P_{350}). The shift in compensation point is considerable, Γ increasing at midday to approx. 180 μ bar. Because stomatal closure occurs simultaneously, P_i remains constant. Ball and Farquhar (1984a, b) reported a very different situation in response to salinity stress with mangrove species. In mangrove leaves, CE remains constant when the photosynthetic capacity decreases and stomatal closure is accompanied by decreasing P_i . The carboxylation efficiency of *Q. suber* at 38°C ($\Delta W = 50$ mbar bar⁻¹) is only 30–40% of that found at 25°C ($\Delta W = 25$ mbar bar⁻¹). This result is not compatible with the conclusion that the temperature dependence of CE is determined by the kinetic constants of ribulose-1,5-bisphosphate carboxylase (RuBPCase)-oxygenase and their respective temperature dependencies (Farquhar et al. 1980; Farquhar and von Caemmerer 1982), if we are to believe, that the temperature dependencies of the kinetic constants are the same for RuBPCase from all plant leaves. Detailed investigation is required to explain why CE decreases with temperature in this species, in contrast to those studies reported previously (Ku and Edwards 1977; Peisker et al. 1979). Since the estimates of CE reported in this paper were determined using time courses with continuous changes in light intensity, temperature, and humidity, the suggestion might be made that time effects or inaccurate determinations of water exchange contribute to the shift found in CE. Steady-state determinations of NP and CE in the laboratory using single leaves of potted *Q. suber* plants, in which extreme care was taken to determine accurately transpiration rate and CO₂ pressure external to the leaf and in which the bypass trapping system was removed, have indicated a similar response

of CE and leaf CO₂ compensation point to increasing temperature and ΔW to that shown in Fig. 7.

Von Caemmerer and Farquhar have emphasized that leaf photosynthesis is regulated to balance RuBPCase activity and RuBP regeneration capacity (von Caemmerer and Farquhar 1981). With *Phaseolus vulgaris*, they found that P_i was regulated in such a manner that an operating point was established relatively high on the CO₂ response curve in the curvature region. They have interpreted this response-curve portion as a transition region between limitation caused by carboxylation capacity and one caused by RuBP regeneration capacity. We have attempted to define the operating point on the CO₂ response curve quantitatively by using the ratio of the photosynthesis rates obtained in normal air (P₃₅₀) and in air with high CO₂ partial pressure (P_{saturation}). In contrast to the results reported by von Caemmerer and Farquhar, data obtained with *Q. suber* leaves indicate an operating point low on the CO₂ response curve. Maximum values of P₃₅₀/P_{saturation} were approx. 0.45 and were obtained on a "standard" day at times of maximal ambient photosynthesis rates in the morning and in the afternoon. It is also at this time that the CO₂-saturated photosynthesis rate becomes light saturated. Values of P₃₅₀/P_{saturation} at midday were as low as 0.15 (Fig. 5) due to a proportionally greater decrease in P₃₅₀ compared with P_{saturation}. The steady-state CO₂ response obtained for midday conditions also provided a low value of 0.27 (Fig. 4). We surveyed results obtained by other investigators by searching the literature for response curves based on internal CO₂, measured to CO₂ saturation, and for which the ambient net photosynthesis rate was given. From 33 such response curves, the average P_{ambient}/P_{saturation} was 0.69 and values ranged from 0.42 to 0.99. Most of these response curves were obtained at relatively low temperatures.

Sharkey (1984) found that a decrease in humidity can depress photosynthesis of leaves but that the total evaporation from the plant had no effect on individual leaves. Sharkey concluded that localized changes in water deficit must develop over small areas within the leaf and regulate the response. Resemann and Raschke have recently found with *Arbutus unedo* a similar strong humidity-induced depression of photosynthesis which appears to play a major role in midday depression (personal communication; Pflanzenphysiologisches Institut der Universität, Göttingen, FRG). Our own experiments with twigs of sclerophylls excised under water and the leaves of which were exposed to "standard" day conditions, indicated

that leaf gas exchange behaves the same as when measured with intact branches. It seems, therefore, probable that the changes in photosynthetic capacity at CO₂ saturation, CE, and Γ described here occur in response to the immediate leaf environment and are controlled independently in individual leaves. Raschke (1982) has reported that in certain situations, application of the stress hormone abscisic acid to leaves of both monocotyledonous and dicotyledonous plants can lead to depression of photosynthetic capacity, decrease in CE, simultaneous stomatal closure, and a constant or slightly elevated P_i. It is possible that a hormonal regulation of this type, which affects photosynthetic characteristics as well as stomatal movement in sequence or in parallel may occur during midday depression. If so, the mechanism by which high temperature and low humidity are sensed is an extremely rapid one which leads to complex, well-coordinated, reversible metabolic changes. Many aspects of the changes in photosynthesis described remain to be examined in further detail. Experiments are planned to separate the influences of temperature and humidity on photosynthesis during midday depression of CO₂ exchange.

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References

- Badger, M., Collatz, G.J. (1977) Studies on the kinetic mechanism of ribulose-1,5-biphosphate carboxylase and oxygenase reactions, with particular reference to the effect of temperature on kinetic parameters. *Carnegie Inst. Washington Yearb.* **76**, 355-361
- Ball, M.C., Farquhar, G.D. (1984a) Photosynthetic and stomatal responses of the grey mangrove, *Avicennia marina*, to transient salinity conditions. *Plant Physiol.* **74**, 7-11
- Ball, M.C., Farquhar, G.D. (1984b) Photosynthetic and stomatal responses to two mangrove species, *Aegiceras corniculatum* and *Avicennia marina*, to long term salinity and humidity conditions. *Plant Physiol.* **74**, 1-6
- Ball, J.T., Berry, J.A. (1982) The C_i/C_s ratio: a basis for predicting stomatal control of photosynthesis. *Carnegie Inst. Washington Yearb.* **81**, 88-92
- Björkman, O., Badger, M., Armond, P.A. (1978) Thermal acclimation of photosynthesis: effect of growth temperature on photosynthetic characteristics and components of the photosynthetic apparatus in *Nerium oleander*. *Carnegie Inst. Washington Yearb.* **77**, 262-282
- Björkman, O., Powles, S.B., Fork, D.C., Öquist, G. (1981) Interaction between high irradiance and water stress on photo-

- synthetic reactions in *Nerium oleander*. Carnegie Inst. Washington Yearb. **80**, 57–59
- Braun-Blanquet, J. (1952) Les groupements végétaux de la France méditerranéenne. Centre National de la Recherche Scientifique, Montpellier
- Cowan, I.R., Farquhar, G.D. (1977) Stomatal function in relation to leaf metabolism and environment. In: Integration of activity in the higher plant, pp. 471–505, Jennings, D.H., ed. Cambridge University Press, Cambridge
- Cowan, I.R. (1982) Regulation of water use in relation to carbon gain in higher plants. In: Encyclopedia of plant physiology, N.S., vol. 12B: Physiological plant ecology II, pp. 589–613, Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H., eds. Springer, Berlin Heidelberg New York
- Dunn, E.L. (1975) Environmental stresses and inherent limitations affecting CO₂ exchange in evergreen sclerophylls in mediterranean climates. In: Ecological studies, vol. 12: Perspectives of biophysical ecology, pp. 159–181, Gates, D.M., Schmerl, R.B., eds. Springer, Berlin Heidelberg New York
- Eckardt, F.E., Heim, G., Methy, M., Sauvezon, R. (1975) Interception de l'énergie rayonnante, échanges gazeux et croissance dans une forêt méditerranéenne à feuillage persistant (*Quercetum ilicis*). Photosynthetica **9**, 145–156
- Farquhar, G.D. (1978) Feed-forward responses of stomata to humidity. Aust. J. Plant Physiol. **5**, 787–800
- Farquhar, G.D., von Caemmerer, S., Berry, J.A. (1980) A biochemical model of photosynthetic CO₂ assimilation in leaves of C₃ species. Planta **149**, 78–90
- Farquhar, G.D., von Caemmerer, S. (1982) Modelling of photosynthetic response to environmental conditions. In: Encyclopedia of plant physiology, N.S., vol. 12B: Physiological plant ecology II, pp. 549–587, Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H., eds. Springer, Berlin Heidelberg New York
- Guttenberg, H., Bühr, H. (1935) Studien über die Assimilation und Atmung mediterraner Macchiapflanzen während der Regen- und Trockenzeit. Planta **24**, 163–265
- Hall, A.E., Schulze, E.-D., Lange, O.L. (1976) Current perspectives of steady-state stomatal responses to environment. In: Ecological studies, vol. 19: Water and plant life, pp. 169–188, Lange, O.L., Kappen, L., Schulze, E.-D., eds. Springer, Berlin Heidelberg New York
- Hellmuth, E. (1971) Eco-physiological studies on plants in arid and semi-arid regions in Western Australia. III. Comparative studies on photosynthesis, respiration and water relations of ten arid zone and two semi-arid zone plants under winter and late summer climatic conditions. J. Ecol. **59**, 225–259
- Ku, S.B., Edwards, G. (1977) Oxygen inhibition of photosynthesis. II. Kinetic characteristics as affected by temperature. Plant Physiol. **59**, 991–999
- Lange, O.L., Meyer, A. (1979) Mittäglicher Stomatenschluss bei Aprikose (*Prunus armeniaca*) und Wein (*Vitis vinifera*) im Freiland trotz guter Bodenwasserversorgung. Flora (Jena) **168**, 511–528
- Lange, O.L., Schulze, E.-D., Kappen, L., Buschbom, U., Evenari, M. (1975) Photosynthesis of desert plants as influenced by internal and external factors. In: Ecological studies, vol. 12: Perspectives of biophysical ecology, pp. 121–143, Gates, D.M., Schmerl, R.B., eds. Springer, Berlin Heidelberg New York
- Lange, O.L., Tenhunen, J.D., Braun, M. (1982) Midday stomatal closure in mediterranean type sclerophylls under simulated habitat conditions in an environmental chamber. I. Comparison of the behavior of various European Mediterranean species. Flora (Jena) **172**, 563–579
- Mahall, B., Schlesinger, W. (1982) Effects of irradiance on growth, photosynthesis, and water use efficiency of seedlings of the chaparral shrub, *Ceanothus megacarpus*. Oecologia (Berlin) **54**, 291–299
- Mooney, H.A., Dunn, E.L. (1970) Convergent evolution in mediterranean sclerophyll shrubs. Evolution **24**, 292–303
- Mooney, H.A., Björkman, O., Collatz, G.J. (1978) Photosynthetic acclimation to temperature in the desert shrub *Larrea divaricata* I: carbon dioxide exchange characteristics of intact leaves. Plant Physiol. **61**, 406–410
- Osmond, C.B., Björkman, O. (1972) Simultaneous measurements of oxygen effects on net photosynthesis and glycolate metabolism in C₃ and C₄ species of *Atriplex*. Carnegie Inst. Washington Yearb. **71**, 141–148
- Osmond, C.B., Björkman, O., Anderson, D.J. (1980) Physiological processes in plant ecology. Toward a synthesis with *Atriplex*. (Ecological studies, vol. 36). Springer, Berlin Heidelberg New York
- Peisker, M., Tichá, I., Apel, P. (1979) Variations in the effect of temperature on oxygen dependence of CO₂ gas exchange in wheat leaves. Biochem. Physiol. Pflanz. **174**, 391–397
- Powles, S.B., Critchley, C. (1980) Effect of light intensity during growth on photoinhibition of intact attached bean leaflets. Plant Physiol. **65**, 1181–1187
- Raschke, K. (1975) Simultaneous requirement of carbon dioxide and abscisic acid for stomatal closing in *Xanthium strumarium* L. Planta **125**, 243–259
- Raschke, K. (1982) Involvement of abscisic acid in the regulation of gas exchange: evidence and inconsistencies. In: Plant growth substances 1982, pp. 581–590, Wareing, P.F., ed. Academic Press, London
- Rouschal, E. (1938) Zur Ökologie der Macchien. I. Der sommerliche Wasserhaushalt der Macchienpflanzen. Jahrb. Wiss. Bot. **87**, 436–523
- Scholander, P., Hammel, H., Bradstreet, E., Hemmingsen, E. (1965) Sap pressure in vascular plants. Science **148**, 339–345
- Schulze, E.-D., Hall, A.E. (1982) Stomatal responses, water loss and CO₂ assimilation rates of plants in contrasting environments. In: Encyclopedia of plant physiology, N.S., vol. 12B: Physiological plant ecology II, pp. 181–230, Lange, O.L., Nobel, P.S., Osmond, C.B., Ziegler, H., eds. Springer, Berlin Heidelberg New York
- Schulze, E.-D., Küppers, M. (1979) Short-term and long-term effects of plant water deficits on stomatal response to humidity in *Corylus avellana* L. Planta **146**, 319–326
- Schulze, E.-D., Lange, O.L., Evenari, M., Kappen, L., Buschbom, U. (1974) The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. I. A simulation of the daily time course of stomatal resistance. Oecologia (Berlin) **17**, 159–170
- Schulze, E.-D., Lange, O.L., Evenari, M., Kappen, L., Buschbom, U. (1975a) The role of air humidity and temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. III. The effect on water use efficiency. Oecologia (Berlin) **19**, 303–314
- Schulze, E.-D., Lange, O.L., Kappen, L., Evenari, M., Buschbom, U. (1975b) The role of air humidity and leaf temperature in controlling stomatal resistance of *Prunus armeniaca* L. under desert conditions. II. The significance of leaf water status and internal carbon dioxide concentration. Oecologia (Berlin) **18**, 219–233
- Sharkey, T., Imai, K., Farquhar, G.D., Cowan, I.R. (1982) A direct confirmation of the standard method of estimating intercellular partial pressure of CO₂. Plant Physiol. **69**, 657–659
- Sharkey, T. (1984) Transpiration-induced changes in the photosynthetic capacity of leaves. Planta **160**, 143–150

- Stocker, O. (1956) Die Abhängigkeit der Transpiration von den Umweltfaktoren. In: Handbuch der Pflanzenphysiologie, vol. 3, pp. 436–488, Ruhland, W., ed. Springer, Berlin Göttingen Heidelberg
- Tenhunen, J.D., Lange, O.L., Braun, M. (1981) Midday stomatal closure in mediterranean type sclerophylls under simulated habitat conditions in an environmental chamber. II. Effect of the complex of leaf temperature and air humidity on gas exchange of *Arbutus unedo* and *Quercus ilex*. *Oecologia* (Berlin) **50**, 5–11
- Tenhunen, J.D., Lange, O.L., Braun, M., Meyer, A., Lösch, R., Pereira, J.S. (1980) Midday stomatal closure in *Arbutus unedo* leaves in a natural macchia and under simulated habitat conditions in an environmental chamber. *Oecologia* (Berlin) **47**, 365–367
- Tenhunen, J.D., Lange, O.L., Jahner, D. (1982) The control by atmospheric factors and water stress of midday stomatal closure in *Arbutus unedo* growing in a natural macchia. *Oecologia* (Berlin) **55**, 165–169
- von Caemmerer, S., Farquhar, G.D. (1981) Some relationships between the biochemistry of photosynthesis and the gas exchange of leaves. *Planta* **153**, 376–387
- Weis, E. (1981) Reversible heat inactivation of the Calvin cycle: a possible mechanism of temperature regulation of photosynthesis. *Planta* **151**, 33–39

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