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The midday depression of CO_2 assimilation in leaves of *Arbutus unedo* L.: diurnal changes in photosynthetic capacity related to changes in temperature and humidity

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Abstract. Parts of attached leaves of the sclerophyllous shrub *Arbutus unedo* were subjected to simulated mediterranean days. Gas exchange was recorded in order to recognize the causes of the midday depression in CO_2 assimilation. Depressions could be induced in part of a leaf: they were local responses. The CO_2-saturation curves of photosynthesis, determined during the morning and afternoon maxima of CO_2 assimilation and during the minimum at midday, established that depressions in CO_2 assimilation were in one-half of the investigated cases totally caused by reversible reductions in the photosynthetic capacity of the leaves, and in the other half almost totally caused by such reductions. An analysis of 37 daily courses showed that morning reductions and afternoon recoveries of stomatal conductance and rate of photosynthesis occurred simultaneously and in proportion to each other, with the result that the partial pressure of CO_2 in the intercellular spaces remained more or less constant. Midday depressions occurred also in detached leaves standing in water. The initiation of a midday depression was not caused by a circadian rhythm, nor was high quantum flux or high temperature a requirement. There was no correlation between the rate of water loss from the leaves, or the amount of water lost, with the degree of reduction of the photosynthetic capacity. However, depressions occurred if an apparent threshold in the water-vapor pressure difference between leaf and air was exceeded. This critical value varied between about 20 and 30 mbar, depending on the leaf investigated. The dominating role of humidity in the induction of the midday depression was further demonstrated when leaf temperature was held constant and the vapor-pressure difference was made to follow the pattern of the mediterranean day: depressions occurred. Depressions however were hardly noticeable when the water-vapor pressure difference was held constant and leaf temperature was allowed to vary. In another set of experiments, leaves were subjected to variations in temperature and humidity independent of the time of the day, under otherwise constant conditions. Photosynthetic capacity and stomatal conductance proved to be almost insensitive to changes in temperature (in a range extending from 20 to 37° C) as long as the water vapor-pressure difference was held constant. If it was not, the rate of photosynthesis began to decline with increasing temperature after a threshold water-vapor pressure difference was exceeded. The position of the resulting apparent temperature optimum of photosynthesis depended on the humidity of the air. We suggest that the ability of *A. unedo* to respond to a dry atmosphere with a reversible reduction of its photosynthetic capacity (by a still unknown mechanism) is the result of a co-evolution with the development of a strong stomatal sensitivity to changes in humidity.

Key words: *Arbutus* – Photosynthesis (midday depression, humidity and temperature dependence) – Stomatal conductance (CO_2 assimilation, midday depression).

Introduction

Some mediterranean shrub species respond to warm and dry weather by closing their stomata during the noon hours; simultaneously, reduction of CO_2 uptake occurs. In the afternoon, stomata reopen and photosynthesis recovers, sometimes to rates equal to those which occurred during the morning maximum. This so-called 'midday depression' in stomatal conductance and CO_2 uptake has been the subject of research ever since 1933 (for a brief historical account see Tenhunen et al. 1984). We became interested in this phenomenon after the discovery that addition of the phytohormone, abscisic acid (ABA), to the transpiration stream of detached leaves also caused simultaneous reductions of stomatal conductance and photosynthetic capacity (Raschke and Hedrich 1985). In leaves supplied with ABA, stomatal and photosynthetic responses were often proportional to each other in magnitude and this resulted in constancy of the intercellular CO_2 concentration. The question arose whether the observed correspondence between short-term stomatal and photosynthetic responses to ABA had been fortuitous, or represented evidence for the evolution of matching response patterns in the stomata and photosynthetic apparatus that would be expressed after application of ABA or during the development of a midday depression.

We investigated whether midday depressions are consequences primarily of stomatal reactions to changing environmental conditions or whether they are manifestations of simultaneous but separate responses of the guard cells on one hand and the photosynthetic apparatus on the other. During the work reported here, we directed our attention mainly to responses of the photosynthetic apparatus to changes in temperature and in the water-vapor pressure differences between leaf and air. As the experimental plant we chose *Arbutus unedo*, because the midday depression had been investigated particularly well in this species, both in the field and in a controlled environment (Lange et al. 1982; Tenhunen et al. 1980, 1981, 1982). Because it was not our objective to provide additional ecophysiological information but rather to analyze photosynthetic responses to elevations in temperature and to dry air, we restricted our work to measurements of the gas exchange of a part of a leaf while the rest of the plant was left under the conditions of the laboratory. This limitation allowed us to produce rapid and well-controlled changes in the air surrounding the leaf and in leaf temperature.

We began our exploration by exposing sample leaves to the conditions of a simulated 'standard mediterranean day' which would cause macchi plants, like *A. unedo*, to respond with midday depressions. Following this procedure of Tenhunen et al. (1980, 1981), we changed the quantum flux, temperature and humidity of the air according to a standardized pattern. At later stages of our research we modified the daily course. In the third phase of our investigation, we determined the effects of changes in temperature and humidity on the photosynthetic capacity independent of the time of the day.

In the context of this report we are using the term ' photosynthetic capacity' for the ability of the leaf to assimilate CO_2 under a particular condition, as it is represented by a particular saturation curve of photosynthesis with respect to the partial pressure of CO_2 in the intercellular spaces of a leaf. A brief report on our work was given on an earlier occasion (Resemann and Raschke 1984).

Material and methods

Plants. Three plants of *Arbutus unedo* L. of unknown age and origin were taken from the Botanical Garden in Göttingen (FRG). They were growing in 10-l tubs in the open in summer and in a cold-house in winter. Eight additional plants were obtained from the Botanical Garden of the University of Würzburg (FRG). They had been propagated from sets brought from Portugal and were of the same origin as the plants used by Tenhunen et al. (1980). These plants were two and three years old and growing in pots of 15 or 20 cm diameter, i.e. root expansion was restricted. During the period of experimentation, November 1982 through August 1983, and in February and March 1984, all plants of *A. unedo* were kept in a greenhouse at temperatures > 15° C. The plants were watered daily, including the day of an experiment.

Leaf chambers and gas analysis. Except when mentioned otherwise, an attached leaf was clamped between two aluminum chambers in each of which an air stream, produced by a small fan, was blown over an area of 6 cm^2 of leaf surface (resulting in a boundary layer conductance of 1.3 $mol \cdot m^{-2} \cdot s^{-1}$ on each leaf side). Leaf temperature was measured by a thermocouple extending along the lower surface of the leaf and was controlled by water circulating through ducts in the chambers and between the glasses of the chamber windows. Light was provided by a xenon arc lamp (XQO 6000 W; Osram, München, FRG) after passage through a Schott UV- and IR-reflecting filter (No. 113 on Tempax glass). A feedback of light, using a silicon photodiode as detector, allowed the irradiance to be held constant at the various desired levels (controlled power supply by Heinzinger, Rosenheim, FRG). The CO_2 content of the air was adjusted by bleeding 5% (v/v) CO_2 in air into a stream of CO_2-free air and monitoring the partial pressure of CO_2 with an infrared gas analyzer. The partial pressure of water vapor in the air was set to desired values by passage of the air through a copper coil submerged into a constant-temperature circulator after humidification at a higher temperature than that of the circulator. The temperature of the copper coil was determined with a thermocouple and the air pressure at the point of condensation of the water by taps before and after the coil, the taps being connected alternatingly to a pressure transducer. For all practical purposes the air pressure in the leaf chambers was equal to the pressure of the ambient air. Atmospheric pressure was monitored with mercury and piezoelectric barometers. Computation of the partial pressures of water vapor in the leaf chambers included corrections for the drop in total pressure between condenser and leaf chambers and for interactions in the ternary system of gas exchange (Caemmerer and Farquhar 1981). The two air streams leaving

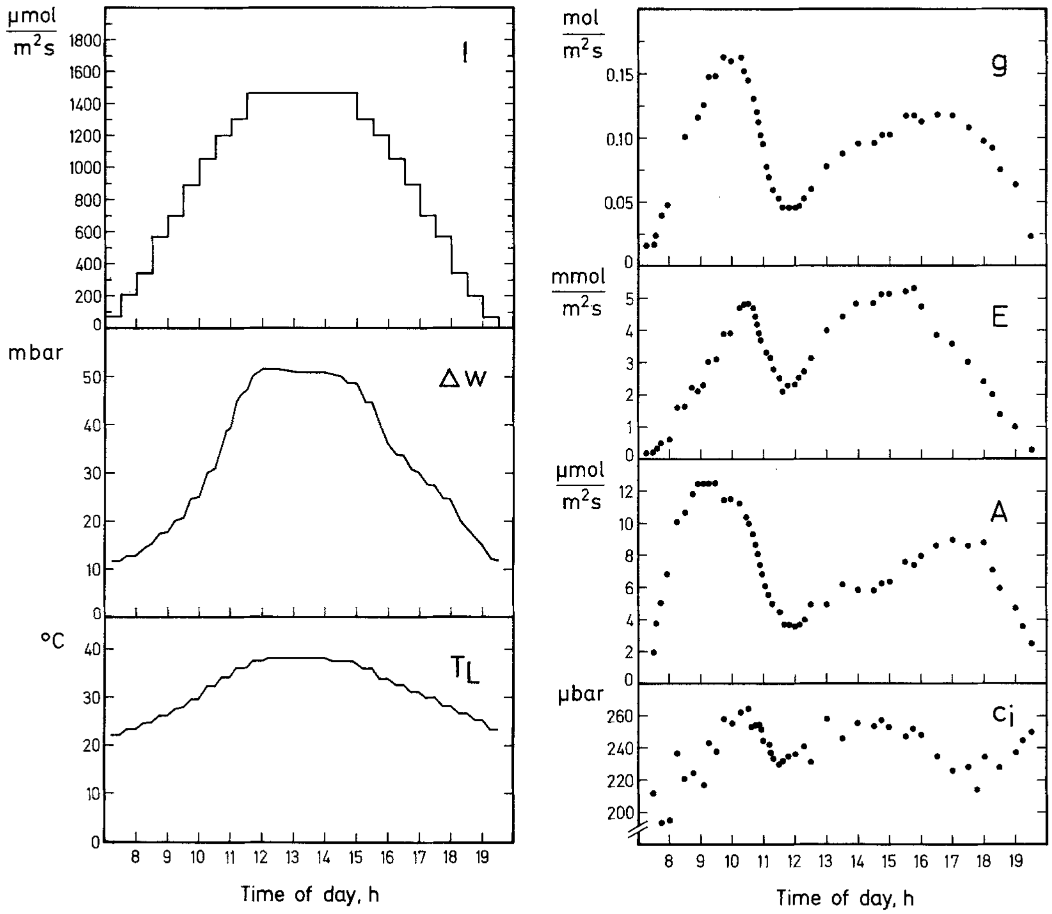

Fig. 1. Response of an attached leaf of *Arbutus unedo* to a simulated mediterranean standard day. *I*, quantum flux; Δw, difference between the water vapor pressure in the leaf and in the air; T_L, leaf temperature; *g*, stomatal conductance for water vapor; *E*, evaporation rate; *A*, rate of CO_2 assimilation; c_i, partial pressure of CO_2 in the intercellular spaces. The partial pressure of CO_2 in the atmosphere, c_a, was between 350 and 370 µbar, that of H_2O was 16 mbar

the upper and the lower leaf chamber were combined and sent through an infrared gas analyzer for H_2O and CO_2 (Binos 1; Leybold-Heraeus, Hanau, FRG). The reference stream of this differential analyzer was also passed through a temperature-controlled leaf chamber, but with a piece of Parafilm in place of the leaf, in order to compensate for transient adsorptions and desorptions of water on the walls of the chambers which occurred whenever temperatures or humidities or both were changed. This procedure could be followed because the change in water-vapor pressure, resulting from the passage of an air stream over a leaf, was usually less than one-tenth of the humidity steps which were imposed to simulate a mediterranean day (Fig. 1).

Results

Reproduction of midday depressions in individual leaves. We first determined whether the phenomenon of a midday depression would occur if only part of a leaf was subjected to the environmental conditions which Tenhunen et al. 1981 described as causing depressions in whole plants. During the morning of a standard mediterranean day, quantum flux and the temperature of the leaf chamber were raised every 30 min in steps, until a quantum flux of 1460 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and a chamber temperature of 35° C had been reached (Fig. 1). Because of the radiation load, leaf temperature had then risen to 38° C. The partial pressure of water vapor in the air remained constant (at 16 mbar) during the course of the day. The water-vapor pressure difference between leaf and air (Δw in Fig. 1) therefore increased from 11 to 51 mbar as the leaf temperature rose. After maintenance of the midday conditions for 3.5 h, quantum flux and chamber temperature were reduced, again in 30-min steps. The assimilation rate began to decline when a vapor-pressure difference of 20 mbar was exceeded, and the leaf temperature was at 28° C. A decrease of stomatal conductance set in later, at a vapor-pressure difference of 30 mbar and a leaf temperature of 32° C. After transition of minima, stomatal conductance and the rate of

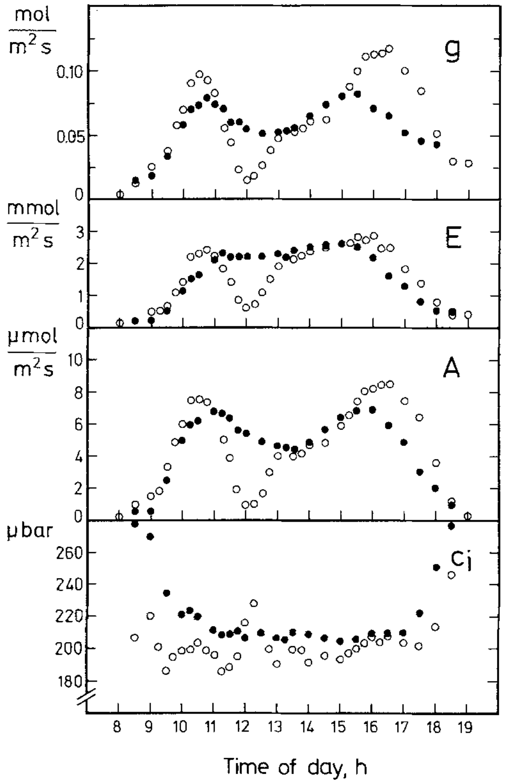

Fig. 2. Responses of an *Arbutus* leaf during simulated mediterranean standard days before (o o) and after (● ●) severance from the plant (done under water). Conditions as in **Fig. 1** except $c_a = 320$–330 µbar (attached leaf) or 340–350 µbar (detached leaf)

CO_2 uptake increased to afternoon peaks. The example shown in Fig. 1 demonstrates that it was possible to induce midday depressions in stomatal conductance and CO_2 assimilation in a part of a leaf while the remainder of the leaf was shaded and the rest of the plant was kept in the temperate environment of the laboratory. A temporal component appeared to be involved in the induction of a midday depression: recovery of photosynthesis and reopening of stomata set in before leaf temperature had reached its peak and when the water-vapor pressure difference was still at its highest value (Fig. 1).

Midday depressions occurred even in detached leaves to which water supply was believed to have been optimal (Fig. 2, solid symbols). However, in detached leaves, midday depressions were not as deep as in leaves on the plant.

Declines and recoveries in photosynthesis and in stomatal conductance were often simultaneous, and the amplitudes of the responses of the photosynthetic apparatus and of the stomata were roughly proportional to each other. Changes in the intercellular CO_2 concentration were therefore much smaller than they would have been if either the stomata or the photosynthetic apparatus had responded alone. Of the 37 midday depressions we investigated, 19 were accompanied by reductions in the intercellular partial pressure of CO_2 between 10 and 30 µbar; in 4 instances the level of CO_2 in the leaves rose by between 7 and 20 µbar, and in the remaining 14 cases the intercellular partial pressure of CO_2 remained nearly constant (within 5 µbar of the morning values). We concluded that, in leaves of *A. unedo*, the midday depression in CO_2 assimilation was not caused by throttled CO_2 supply to the mesophyll but rather by an inhibition of the photosynthetic apparatus itself.

The magnitude of the reductions in CO_2 assimilation (and in stomatal conductance) varied widely among the leaves: the observed minima were between 0.3 and 0.9 of the maxima recorded during the morning. In most cases, stomatal closure was strong enough to cause reductions in water loss (Figs. 1 and 2), overcompensating the steep increases in the gradients of water-vapor pressure between leaf and air which occurred during the simulated mediterranean days.

Diurnal changes in photosynthetic capacity. The conclusion that the photosynthetic apparatus had been directly struck during a midday depression needed unequivocal evidence. Saturation curves of assimilation with respect to CO_2 were determined during three conspicuous phases of photosynthetic behavior: during the maximum in the morning, during the greatest depression at midday, and after full recovery in the afternoon. Because it took about an hour to complete the measurements required for the construction of a saturation curve, the standard day was modified by stopping the temperature rise at 32° C, when the quantum flux was at 830 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and keeping these conditions before the afternoon decline was initiated. This modification had no serious consequences because the midday depression was already fully expressed at the 32° C level. In all of the four replications of the experiment, the saturation curve taken during the depression showed a lowered initial slope, a lowered maximal rate, and a rise of the CO_2 compensation point (Fig. 3). In each case, the photosynthetic apparatus was able to recover fully from the depression. In the example shown in Fig. 3 (and in the attached leaf of Fig. 2), the photosynthetic capacity was even higher in the afternoon than in the morning.

Stomatal responses to CO_2. Not only did the relationship between the rate of photosynthesis and the partial pressure of CO_2 in the leaf change when a midday depression occurred, stomatal behavior was also modified. In the morning, before the development of a depression, stomatal conductance was virtually independent of the partial pressure of CO_2; the same was true in the afternoon. During the noon hours, however, when a midday depression had developed, stomata were sensitive to CO_2 (Fig. 4).

High temperature and low humidity as causes of the midday depression. In the 37 diurnal courses we followed during this investigation, midday depressions were initiated after leaf temperatures between 29 and 35° C and water-vapor pressure differences between 25 and 35 mbar had been exceeded. In order to recognize the contributions made by temperature and humidity to the development of a midday depression, minimum assimilation rates were plotted against leaf temperature and against the water-vapor pressure difference that occurred at the onset of the morning decline. Plots were also made against values of these two variables as they were measured at the time of the midday minimum. No correlations emerged between minimum assimilation rate and leaf temperature, or vapor pressure difference for that matter, at the beginning of the depression (not shown), and none with leaf temperature at the time of the minimum either (Fig. 5, lower panel). There was an indication of a correlation with the water-vapor pressure difference at the time of the minimum (Fig. 5, upper panel). Linear regression of the data showed that a negative correlation between the rate of assimilation and the water-vapor pressure difference during the midday minimum could account for 44% of the observed decline in photosynthesis; rising leaf temperature could account for not more than 14% (legend to Fig. 5). Stomatal behavior corresponded to that of the photosynthetic apparatus in all cases and is therefore not documented here.

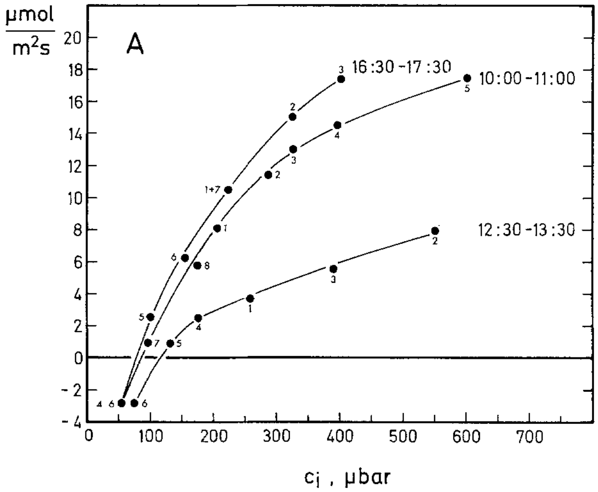

Fig. 3. The CO_2 saturation of photosynthesis in an attached *Arbutus* leaf, determined during the morning maximum (10:00–11:00 h), the midday depression (12:30–13:30 h), and the afternoon maximum (16:30–17:30 h) of one and the same day. In the morning $T_L = 27.5°$ C, $\Delta w = 21$ mbar; during midday $T_L = 32°$ C, $\Delta w = 32$ mbar; in the afternoon $T_L = 26°$ C, $\Delta w = 19$ mbar. Quantum flux $I = 830$ µmol·m^{-2}·s^{-1} throughout. Numbers indicate the sequence in which the measurements were made

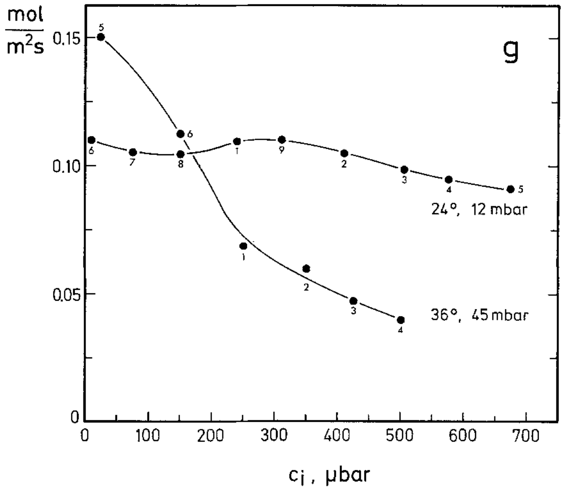

Fig. 4. Relationships between conductance for water vapor, g, of an attached *Arbutus* leaf and the partial pressure of CO_2, c_i, in the intercellular spaces under morning conditions ($T_L = 24°$ C, $\Delta w = 12$ mbar) and during a midday depression ($T_L = 36°$ C, $\Delta w = 45$ mbar). Quantum flux $I = 1500$ µmol·m^{-2}·s^{-1}. Numbers indicate the sequence in which the measurements were made

Correlation of the minimum assimilation rate with evaporation rate or its integral with time. It is conceivable that midday depressions had been caused by excessive rates of water loss; alternatively they could have been triggered after a critical amount of water had been lost. We related minimum rates of CO_2 assimilation to rates of water loss at the beginning of the depressions and also to the amounts of water lost during the time span from exposure to light to the onset of a decline in photosynthesis (Fig. 6). We could not recognize any correlation of the minimum rate of assimilation with rates of transpiration (Fig. 6, top panel), nor could we detect that depressions could be corrective responses that occurred after presumed critical amounts of water had been lost (Fig. 6, bottom panel). As a matter of fact, if a correlation appeared, it was a positive one, indicating that assimilation increased with the amount of water lost.

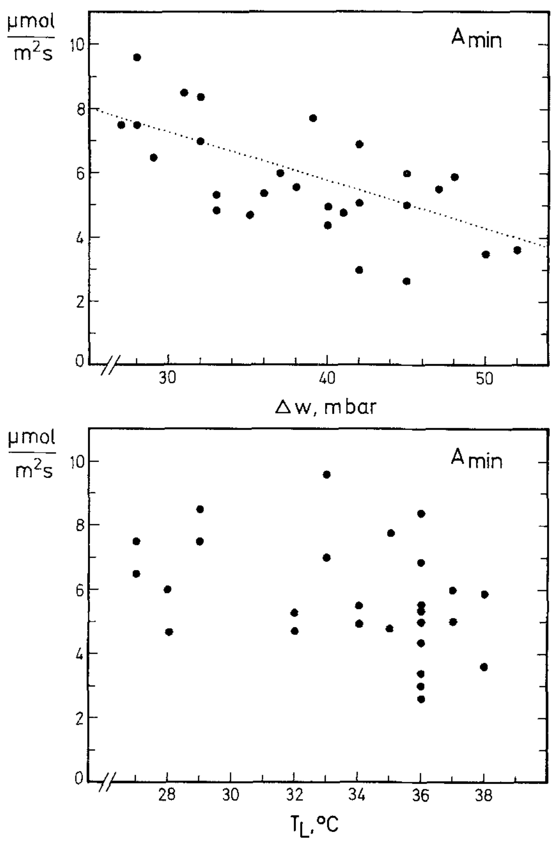

Fig. 5. Rates of CO_2 assimilation of 27 *Arbutus* leaves, as determined during the midday minimum, plotted against the water-vapor pressure difference between leaf and air (*top*) or the leaf temperature (*bottom*) as they were measured during the minimum. For a reduction of scatter, only those experiments were included in the analysis in which the assimilation rate was $\geq 7.4\ \mu mol \cdot m^{-2} \cdot s^{-1}$ during the morning peak. Linear regression of the data for $\Delta w \geq 25$ mbar and $T_L = \geq 25°$ C resulted in $A_{min} = 12.1–0.162\ \Delta w$ (*dotted line*), $r^2 = 0.44$; and $A_{min} = 12.1–0.188\ T_L$, $r^2 = 0.14$

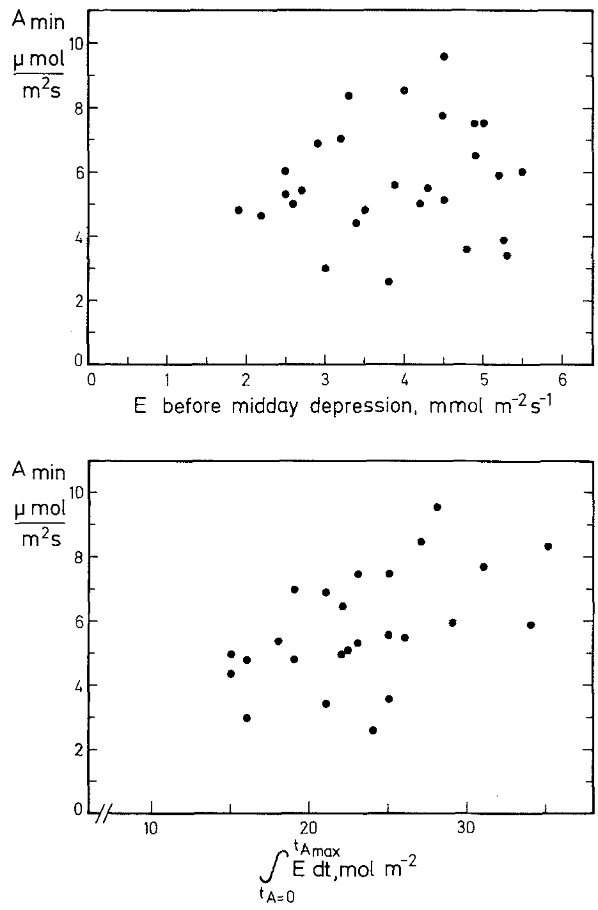

Fig. 6. Rates of CO_2 assimilation during the midday minimum related to the rate of evaporation at the onset of a depression (*top*) or the amount of water lost between the beginning of the light period and the onset of a depression (*bottom*). From 27 and 25 leaves, respectively, subjected to mediterranean standard days and with $A \geq 7.4\ \mu mol \cdot m^{-2} \cdot s^{-1}$ during the morning peak

This finding merely indicated that stomatal conductance was large, when a leaf's photosynthetic capacity was high. There was no evidence for high rates of water loss causing declines in photosynthesis.

Diurnal records of gas exchange when leaf temperature or humidity gradient was held constant. In order to increase the resolution of our experimental procedure and to gain time available for scanning leaf behaviour during the course of a day, the severity of the environmental conditions was reduced during the following experiments. The ceiling for quantum flux was lowered from $I = 1460$ to 900 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and that of maximum leaf temperature from $T_L = 38$ to 34° C. As a consequence, water-vapor pressure differences rose to only about 37 mbar. Nevertheless, midday depressions were induced (Fig. 7). The extent of the reductions in assimilation was of a magnitude similar to that reached on a standard day. We learned from this modification (and other experiments we do not describe here) that high irradiance was not a requirement for the induction of a midday depression (it could occur at a quantum flux as low as 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$). During another moderated day, the temperature of the leaf of Fig. 7 was held constant, while stepwise variations in the water-vapor pressure difference were repeated (Fig. 8). On the following day the water-vapor pressure difference was kept constant as well (also in Fig. 8). A depression in CO_2 assimilation set in after the vapor-pressure difference increased beyond 20 mbar; obviously, a rise in leaf temperature was not required for the formation of a depression. Virtually no depression of assimilation was recorded when the vapor-pressure difference remained unchanged (at 13 mbar). The experiment

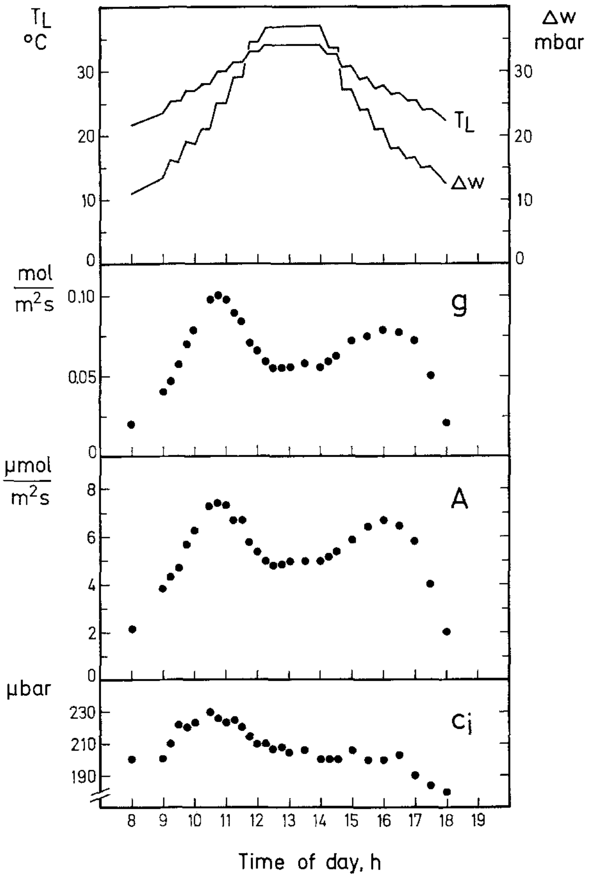

Fig. 7. Expression of a midday depression on stomatal conductance and CO_2 assimilation of an attached *Arbutus* leaf at reduced quantum flux ($I = 900\ \mu mol \cdot m^{-2} \cdot s^{-1}$ during midday) and reduced maximum leaf temperature, $T_L = 34°$ C, but with the water-vapor pressure difference, Δw, exceeding 30 mbar during midday. This is the reference day for the experiments shown in **Figs. 8** and **9**. Same symbols as in **Fig. 1**

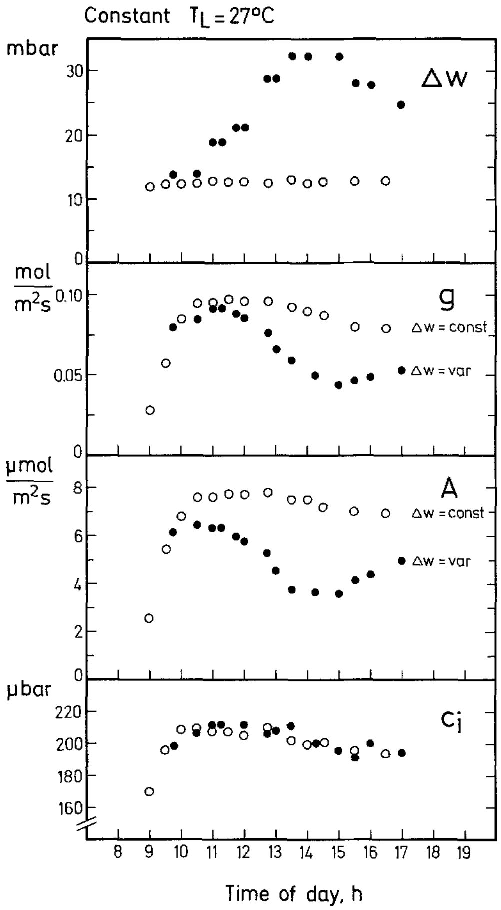

Fig. 8. The same attached leaf as that used for the experiment of **Fig. 7** was subjected to stepwise changes in the water-vapor pressure difference, Δw, between leaf and air, while leaf temperature, T_L, was kept constant at27° C (● ●). A control experiment at constant $\Delta w = 13$ mbar and constant $T_L = 27°$ C was performed on the following day (o o). I during midday = $900\ \mu mol \cdot m^{-2} \cdot s^{-1}$

was continued, still with the same leaf, with the objective to assess the magnitude of any possible minor contribution made by temperature to the expression of the midday decline (Fig. 9). In this experiment, the dew point of the air was adjusted to changes in leaf temperature in a way that the water-vapor pressure difference remained close to 15 mbar. A small depression began to form only after the temperature had risen to 31.5° C. When leaf temperature had reached 34.5° C, assimilation rate had declined by 12% of the maximum rate of the day. This reduction was smaller than the 35% reduction that had occurred in the same leaf during the modified standard day at the same leaf temperature but at a larger vapor-pressure difference ($\Delta w = 37$ mbar; Fig. 7). It was also smaller than the 44% reduction which was recorded at a constant leaf temperature of 27° C and a vapor-pressure difference of 32 mbar (Fig. 8). In the induction of the midday depression, effects of temperature on the photosynthetic apparatus appear to be subordinate to events following increases of the water-vapor pressure difference between leaf and air.

Effects of temperature and humidity on the photosynthetic capacity. We tested the primary role of humidity in causing the midday decline by determining saturation curves of the assimilation rate with respect to CO_2 during the midday hours. The example shown in Fig. 10 shows that the photosynthetic capacity of a leaf increased when, on subsequent days, leaf temperature was brought to 25, 30 and to 32° C, as long as the difference in water-vapor pressure between leaf and air was adjusted to approx. 15 mbar each time. If this was not done, and the humidity difference was allowed to increase to 32 mbar with rising temperature, photo-

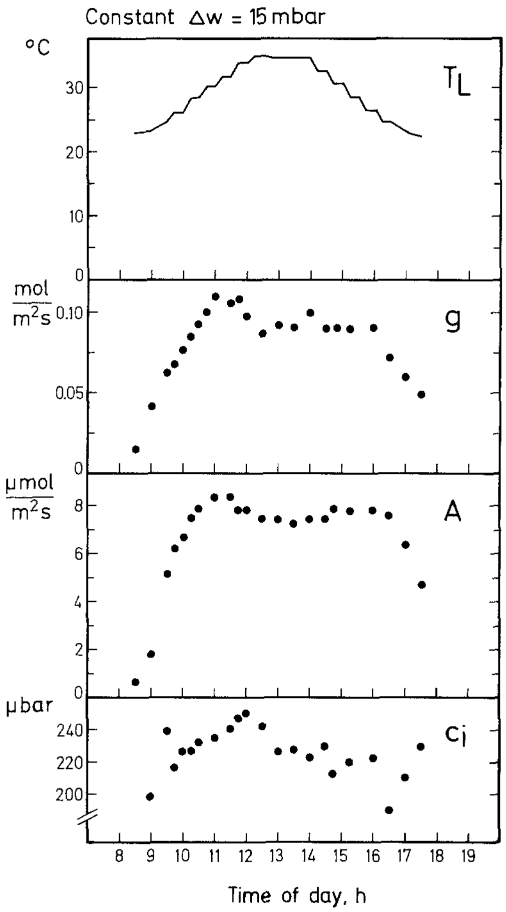

Fig. 9. The same attached leaf as that used for the experiments of **Figs. 7** and **8** was subjected to stepwise changes in leaf temperature, T_L, while the water-vapor pressure difference between leaf and air was kept constant at $\Delta w = 15$ mbar by changing the dew point of the air. I during midday $= 900$ µmol·m^{-2}·s^{-1}

synthetic capacity declined to one quarter of the high-humidity value, rather than increased with temperature (at an intercellular partial pressure of $CO_2 > 200$ µbar).

The time courses of assimilation responses to large increases in the water-vapor pressure difference followed a pattern typical for the phase of decline of a midday depression (Figs. 1, 2, 7), also when the temperature was held constant. However, responses were usually twice as fast as during the development of a depression. After application of a step in Δw from 15 to 30 mbar, photosynthesis declined to a new, low equilibrium within 1 h (admittedly with broad variation). Recovery upon a step back from $\Delta w = 30$ to 15 mbar was slower than the decline; usually it took several hours for a complete return to the original rate. Again there was wide variation among leaves in their temporal behavior.

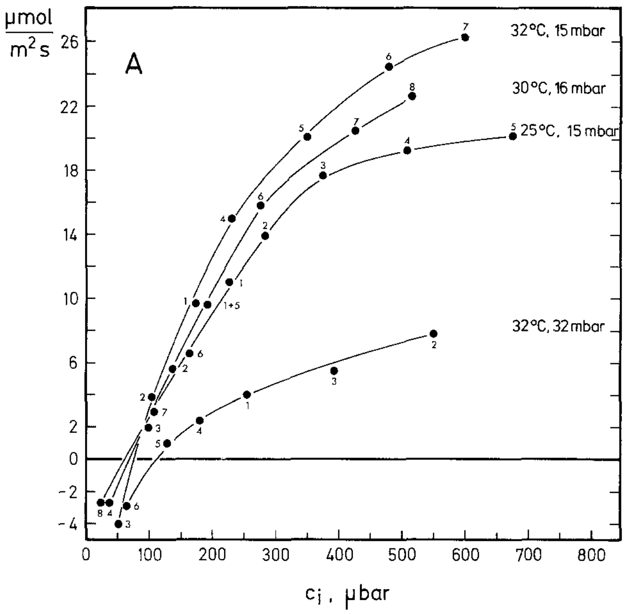

Fig. 10. The CO_2 saturation of photosynthesis of an attached *Arbutus* leaf as modified by temperature and water-vapor pressure difference between leaf and air during midday on subsequent days. One out of two examples. Quantum flux = 830 µmol·m^{-2}·s^{-1}. Numbers indicate the sequence in which the measurements were made

The importance of the humidity difference in determining the photosynthetic capacity of leaves of *A. unedo* was ascertained in another set of experiments in which the humidity dependence of stomatal conductance and of the assimilation rate was recorded at leaf temperatures of 27, 30, 33, and 35° C. At each temperature level, rates of photosynthesis declined with increasing vapor-pressure difference, particularly if the gradient exceeded about 30 mbar. The effect was expressed most strongly at a leaf temperature of 27° C (Fig. 11). Despite declining assimilation rates, partial pressures of CO_2 in the intercellular spaces remained fairly constant (Fig. 11) because stomata also responded to changes in humidity. Their responses were proportional to the responses of the photosynthetic apparatus and are therefore not presented here.

The dependence of photosynthesis on the vapor-pressure difference was maintained when the water supply to a leaf was improved. When leaves were detached and placed in water they assimilated CO_2 at higher rates than when they were still on the plant. However, assimilation rates of detached leaves remained sensitive to increasing vapor-pressure differences; Fig. 12 shows an example. After this leaf was cut from the plant it was weighed three times: before and after gas analysis, and after standing in water for one night. The leaf's weight increased during gas analysis but not any further during the following night, showing that exposure

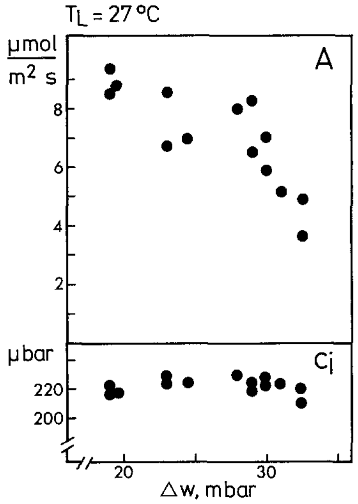

Fig. 11. Assimilation rates (*A*) and intercellular partial pressures of CO_2 (c_i) of 14 attached *Arbutus* leaves kept at a leaf temperature of 27° C and exposed to various differences in water-vapor pressure between leaf and air (Δw). Quantum flux = 1 000 μmol·m^{-2}·s^{-1}

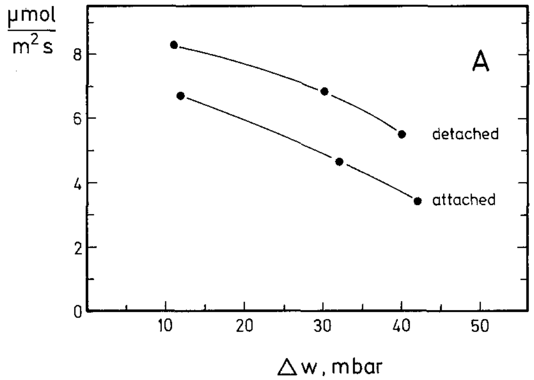

Fig. 12. Dependence of CO_2 assimilation (*A*) on an increasing water-vapor pressure difference between leaf and air (Δw) in an attached leaf and in the same leaf on the following day after separation from the plant. The leaf gained water during the measurement of the gas exchange in the detached state. T_L = 31° C; c_a = 370 μbar (attached) or 350 μbar (detached); I = 900 μmol·m^{-2}·s^{-1}

to large vapor-pressure differences did not lower the bulk water content of the tissue. Two additional experiments confirmed this finding.

Dependence of the optimum temperature for photosynthesis on humidity. In order to separate effects of temperature on photosynthesis from effects of the water-vapor pressure difference, temperature dependences of photosynthesis were determined in the range extending from 20 to 37° C, while vapor-pressure differences were held constant at various values between 8 and 32 mbar. In contrast to the experiment of Fig. 10, temperature treatments were not spread over the 'depression hours' of subsequent days but applied as temperature scans extending over a few hours on each day of experimentation. In nine out of the 13 individual leaves we tested, temperature-dependent changes in CO_2 assimilation and stomatal conductance remained within 10% of their mean values for the investigated temperature range. Three examples for temperature dependence of CO_2 assimilation are shown in Fig. 13. It was in only two of the 13 leaves that assimilation declined when leaf temperature was raised above 30° C. In leaves of *A. unedo*, the mechanisms of CO_2 assimilation and of the stomata seemed to be temperature-compensated. Temperature dependences of assimilation however appeared if the vapor-pressure difference was allowed to increase as leaf temperature rose (Fig. 14). The emerging temperature optima of CO_2 assimilation were only apparent. Their position depended on the magnitude of the water-vapor pressure difference between leaf and air. In the example shown in Fig. 14, photosynthesis declined after a vapor-pressure difference of 18 to 20 mbar was exceeded. This response to humidity produced optima which shifted from 21° C to 24° C, and to 29° C, depending on the water-vapor pressure in the air. That the reductions in photosynthesis shown in Fig. 14 were indeed effects of increasing vapor-pressure gradients, and not direct effects of temperature on the photosynthetic apparatus, could be shown when a further temperature-response curve of the same leaf was determined,

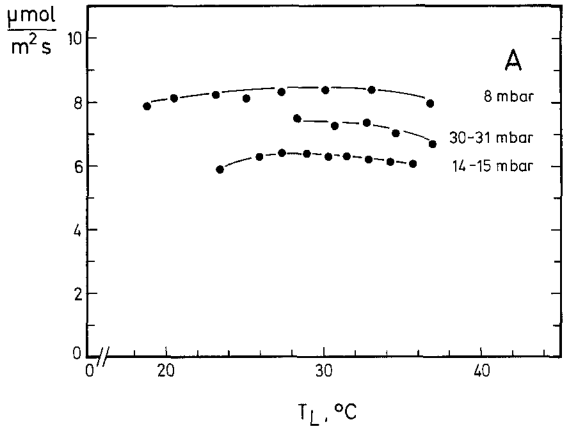

Fig. 13. Temperature dependence of CO_2 assimilation rates of three attached *Arbutus* leaves of differing initial photosynthetic capacities. The water-vapor pressure difference between leaf and air was held at the three indicated levels by adjustment of the dew points of the air entering the leaf chamber. I = 900 μmol·m^{-2}·s^{-1}; c_a = 340 μbar. Sequence of measurements: from lowest to highest T_L

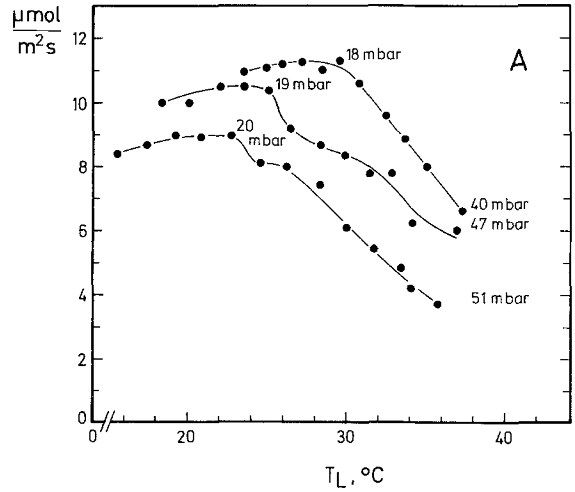

Fig. 14. Temperature dependence of CO_2 assimilation rates of an individual attached *Arbutus* leaf at three constant partial pressures of water vapor in the air. *Upper curve*, 22 mbar; *middle curve*, 12 mbar; *lower curve*, 6 mbar. Numbers on the curves give the water-vapor pressure differences at the nearest data points. $I = 900\ \mu mol \cdot m^{-2} \cdot s^{-1}$; $c_a = 350\ \mu bar$. Sequence of measurements: from lowest to highest T_L

this time at a constant water-vapor pressure difference of 8 mbar: the response was flat; only when the humidity gradient was increased to 40 mbar (at a leaf temperature of 36° C) did the rate of assimilation decline to one-half of its original value (not shown).

Discussion

Reductions in photosynthetic capacity. Our investigation was greatly facilitated by the experience that midday depressions in stomatal conductance and CO_2 assimilation could be reproduced in parts of individual leaves of *A. unedo*. The conclusion of Lange et al. (1982) that stomatal behavior was in this species identical in the field and under simulated mediterranean conditions in a growth chamber can be extended, within obvious limits, to a partial area of an individual leaf. After comparing our results with those of Beyschlag (1984) and Lange et al. (1985) we can add, that the photosynthetic apparatus of *A. unedo* also performs in the laboratory in a way comparable to its behavior in the field.

A reversible reduction in the photosynthetic capacity was the cause of the midday depression in CO_2 uptake. Although stomatal closure occurred simultaneously with the decline in assimilation, and opening was synchronous with recovery (Figs. 1, 2, 7), depressions in stomatal conductance were not the cause of depressions in CO_2 assimilation. Reference to Figs. 3 and 10 illustrates that a reduction of photosynthesis down to one-half of its morning maximum would have required a decline of the intercellular partial pressure of CO_2 from 220 to between 120 and 150 µbar; a decline of this magnitude was never observed (Figs. 1, 2, 7–9, 11). This finding matches the report of Schulze et al. (1975) who estimated that the intercellular CO_2 concentration changed astonishingly little in leaves of *Prunus armeniaca* when stomatal resistance increased in the course of a noon decline and our conclusions find support in the results of Beyschlag (1984), obtained with leaves and branches of *A. unedo*, and in the measurements of Tenhunen et al. (1984) on branches of *Quercus suber*, another mediterranean species undergoing midday depressions. Carbon-dioxide saturation curves of photosynthesis taken during a midday depression resembled curves that were obtained after application of ABA to leaves of herbaceous species (Raschke and Hedrich 1985). Nevertheless, such a similarity is insufficient evidence for an involvement of ABA in the induction of the midday depression.

Low humidity as the primary cause of the midday depression. Leaves of *A. unedo* could stand much higher quantum fluxes (up to $1600\ \mu mol \cdot m^{-2} \cdot s^{-1}$) than they were exposed to during cultivation (200 to 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$), or at which photosynthesis would saturate (500 to 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$, in agreement with observations in the field in Portugal, Lange et al. 1985). High irradiance was not a cause of the midday depression; depressions could be induced at quantum fluxes of 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$. All of our experiments provided evidence for a dominant role of humidity, as Lange et al. (1982) had suspected and Schulze et al. (1972) had found for the stomatal behavior of plants growing in a desert. The small size of the exposed area in the leaf chamber proved to be essential for the success of our experimental analysis because the water-vapor pressure in the leaf had to be determined with high accuracy, and this required leaf temperature to be measured with high accuracy at a representative spot.

That a circadian component was not essential for the development of a midday depression became obvious during the experiments in which mediterranean days were no longer imposed and only one environmental variable changed at a time while all others were held constant. These experiments reinforced the conclusion that humidity preponderated among the causal variables (Figs. 11, 12, 14). In most of the experiments, a decline in photosynthesis set in after a critical vapor pressure difference had been exceeded, usually about 30 mbar (Fig. 11), in agreement with the report by Tenhunen et al. (1982) on the initiation of a midday stomatal closure in *A. unedo*. However, threshold values derived from one experiment did not apply to others, and in still other experiments the decline in the rate of photosynthesis was gradual.

Humidity dependence of the optimum temperature for photosynthesis. Leaves of *A. unedo* were remarkable in their virtual independence of CO_2 assimilation (and stomatal conductance) from temperature within a broad range, extending from 20 to 37° C in one of the cases shown in Fig. 13. This low sensitivity appeared when temperature scans were made on the same day; temperature dependences occurred when treatments were spread over several days: Fig. 10. Biochemical mechanisms, if considered individually and disconnected from the metabolic network of which they are parts, should be strongly temperature dependent. Since we observed in several leaves an almost complete insensitivity to changes in temperature in the assimilation of CO_2 we cannot help but conclude that a mechanism of temperature compensation had evolved in the photosynthetic and the stomatal apparatus of *A. unedo*. We venture the suggestion that this evolution is in its perfection peculiar to this species, and perhaps also to other species which exhibit pronounced midday depressions. If in experiments with these plants, temperature optima of photosynthesis occurred, they were only apparent. In reality, they occurred in response to changes in the water-vapor pressure in the leaf. One should be able to recognize situations in which temperature optima are not genuine. If a temperature optimum is only apparent, its position on the temperature scale should depend on the moisture content of the air. Lange et al. (1985) reported an observation to that effect.

Speculations on paths of control. Explanations of the humidity dependence of the photosynthetic capacity of leaves of *A. unedo* have to take the following facts into consideration. (i) The midday depression is a local phenomenon. (ii) Stomatal and photosynthetic responses are simultaneous (Figs. 2, 7); the decline in photosynthesis sometimes precedes the decline in stomatal conductance slightly (Figs. 1, 8 and 9). (iii) The rate of evaporation usually declines during a midday depression, although the water-vapor pressure gradient is then highest (Figs. 1, 2; Schulze et al. 1972). (iv) The depression has a transient component; recovery sets in while high temperature and a large water-vapor gradient persist (Figs. 1, 2 and Lange et al. 1982). (v) Responses to dry air occur even if water supply is optimal (Fig. 2), independent of the water content of the leaf (experiment of Fig. 12) or the leaf's water potential (Tenhunen et al. 1980). (vi) The amplitude of the midday depression does depend on water supply (Fig. 2) or on the water potential of the leaf tissue (Tenhunen et al. 1982). We know that stomata can respond directly to the humidity of the air, irrespective of the water content of the tissue (Raschke 1970; Lange et al. 1971; Schulze et al. 1972; for a review, see Schulze 1986). The simplest explanation of the humidity dependence of the midday depression would be to postulate that a message is sent from the epidermis to the mesophyll. All observations, except (iv), could be explained by such a course of events, even (ii), if there is some internal delay in the guard cells' response. There is, of course, the question after the messenger. While we are used to the view that ABA moves from the mesophyll to the epidermis, we cannot entirely exclude the possibility of a movement in the opposite direction. This notion is probably not true, but its falsity has not been proven either.

An alternative explanation can be based on the assumption of a sensing current of transpiration through the leaf tissue (similar to a suggestion by Sharkey 1984). This current must bypass stomatal control and cause a (local) change in water potential (or turgor, osmotic pressure, or volume) in a specific compartment in the leaf. From there signals will be sent to the guard cells and simultaneously to the photosynthetic apparatus. This sensing current must be connected, through a separate resistance, to the main transpiration stream, which passes a series of resistances, and gains or loses water by exchange with the tissue as it moves through the leaf (Cowan 1972). The operating point of the sensing mechanism (on a turgor, solute, or volume curve) is thus determined by water loss and supply. The system contains elements which cause delay and thereby contribute a transitory component to the midday depression. The sensing compartment could be within the photosynthetic tissue. Assimilation of CO_2 by leaf slices decreased during osmotic dehydration (Kaiser et al. 1983). Alternatively, ABA could serve as a messenger substance, either after release from the chloroplasts, due to a change in their volume (Hartung et al. 1983), or after synthesis. Burschka et al. (1983) reported that the ABA content of leaves increased during the midday hours of one day in irrigated and non-irrigated plants of *A. unedo,* and injection of ABA into the petiole of an *Arbutus* leaf caused declines in stomatal conductance and net assimilation (Burschka et al. 1985). However, more supporting evidence is required to assign a role to ABA in the expression of the midday decline.

Teleology of humidity control of gas exchange. In magnitude, the humidity dependence of photosynthesis in *A. unedo* goes much beyond the reported effects of dry air on CO_2 assimilation in herbaceous species as they were described, for instance, by Bunce (1984) and Sharkey (1984). The phenomenon also differs from the effect of cultivation at low atmospheric humidity on the photosynthetic capacity of two mangrove species (Ball and Farquhar 1984), although these authors also quoted an observation of a reversible short-term effect on the plateau region of the CO_2-saturation curve of photosynthesis. We presume that the evolution of temperature compensation and humidity dependence in the photosynthetic apparatus of *A. unedo* was the result of a strongly expressed stomatal ability to respond to a dry atmosphere with a double-peaked diurnal activity. Some other mediterranean sclerophylls, but not all of them, seem to share this ability (Lange et al. 1982).

Cowan and Farquhar's (1977) assessment of stomatal function in relation to leaf metabolism and environment led, among several other conclusions, to the postulate that, in a dry environment, an optimization of water loss with respect to carbon uptake will necessitate a reduction of stomatal conductance during the middle of the day. A corresponding reduction of photosynthetic capacity was not required by the theory, and a CO_2 depletion of the intercellular spaces was assumed and tacitly accepted (their Fig. 11). Scarcity of CO_2 could result in an overreduction of the photosynthetic apparatus and an inhibitory acidification of the thylakoids (Kobayashi et al. 1982). Co-evolution of a sensitivity of the photosynthetic apparatus to the same environmental factors which determine the width of the stomata could prevent damage by keeping the partial pressure of CO_2 in the intercellular spaces at normal levels (between about 200 and 260 μbar in leaves of *A. unedo*).

We recognize that diurnal variation in photosynthetic capacity in turn requires an ability of the stomatal system to adjust to changes in CO_2 demand, regulation has gone round the circle. (Possibly, the increased CO_2 sensitivity of the stomata during the noon hours assists in this task, Fig. 4.) Optimal water use requires the quantity $\partial E/\partial A = \lambda$ to remain constant through the course of the day (Cowan and Farquhar 1977). Estimates of λ for the day shown in Fig. 1 yielded a value of $\lambda = 1\,000$ for the morning maximum; it was $\lambda = 1100$ during the midday minimum, and $\lambda = 600$ in the afternoon. Had photosynthetic capacity remained constant, the noon value would have been at $\lambda = 360$ (at $A = 5.4\ \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $c_i = 160\ \mu\text{bar}$). On another day, the morning, noon, and afternoon values had been at $\lambda = 250$, 450, and 250 mol H_2O/mol CO_2. A constant photosynthetic capacity would have resulted in $\lambda = 80$ during the depression. Normally, $\partial E/\partial A$ was between 200 and 500 in the attached leaves of *A. unedo* we investigated.

What could be the utility of a reduced direct sensitivity to temperature, could there be a functional relationship to the high sensitivity to humidity? The rate of water loss did not serve as a signal that called for a midday decline, nor was it the amount of water lost that was gauged by the leaves of *A. unedo.* Obviously, changes in water content would have been too slow for an optimal control of gas exchange. If $\partial E/\partial A = (\partial E/\partial g)/(\partial A/\partial g)$ were to be kept constant during the course of a dry, sunny day with a wide amplitude of the temperature curve, sensitivity to Δw would provide the most direct means to maintain $\partial E/\partial A$, because $\partial E/\partial g \approx \Delta w$. In this context, work of Kirschbaum and Farquhar (1984) is relevant. They modelled the temperature dependence of photosynthesis in eucalypt leaves and came to the conclusion that $\partial E/\partial A$ = constant requires the optimum temperature of net assimilation to decline with decreasing ambient humidity. We recognize that, in leaves of *A. unedo*, the existence of a direct strong temperature dependence of assimilation, one that is additional to the temperature dependence inherent in the humidity response, would complicate regulation. It appears worth further study whether a reduction of the direct temperature sensitivity of the stomata and the photosynthetic apparatus, in combination with the acquisition of a high sensitivity to humidity, is a general trait of species from the sclerophyllous zone.

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