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Effects of Frost on Leaf Gas Exchange and Rubisco Activity in the Subalpine Species *Eucalyptus pauciflora* ssp. pauciflora Sieb. ex Spreng. (Snow Gum)

By

B. I. L. KÜPPERS[1)2)] & M. KÜPPERS[3)4)]

Key words: Australia, Brindabella Ranges, carboxylation efficiency, diurnal courses, frost effects, intercellular CO_2 concentration, leaf conductance, quantum yield, rubisco activity, temperature response, winter-time photosynthesis.

Summary

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Leaves of the evergreen *Eucalyptus pauciflora* (as well as of *E. delegatensis*) were studied at the plants' natural, subalpine field sites. Frost down to -10°C only temporarily affected leaf gas exchange which fully recovered after a few days without frost. Potential rubisco activity was also maintained over the frost period. The response of net photosynthesis to leaf temperature, measured in the field, differed only slightly from that in summer and autumn with a temperature optimum ranging from 20 to 25°C contrasting previous observations from laboratory studies and contrasting the situation in the European Alps.

Introduction

In *Eucalyptus pauciflora*, SLATYER & MORROW 1977 and SLATYER 1978 observed strong differences in the leaf temperature (T_l) response of net photosynthesis (A). Their plants were sampled at different altitudes and different times of the year, cultivated and measured under greenhouse conditions. Therefore

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[1)] Institut für Botanik, TU Darmstadt, D-64287 Darmstadt, Germany.
[2)] Research School of Biological Sciences, ANU, Canberra, ACT 2601, Australia.
[3)] Institut für Botanik, Universität Hohenheim, D-70599 Stuttgart, Germany.
[4)] CSIRO, Div. Forestry Forest Products, Canberra, ACT 2604, Australia.

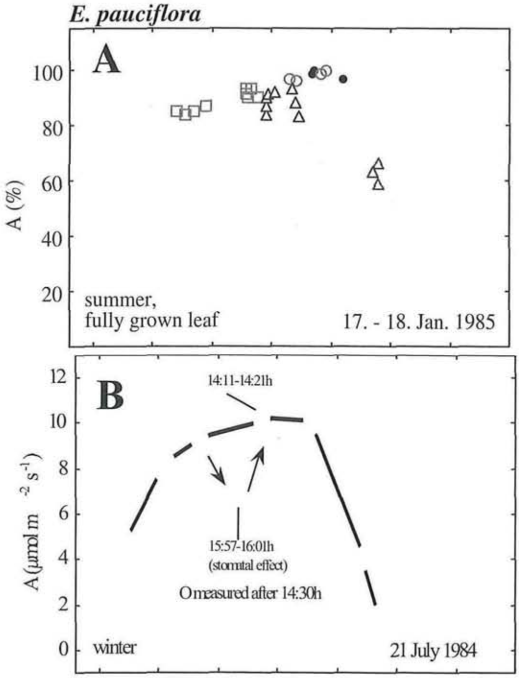
it is not evident, whether such responses also occur at natural field sites. This we studied here relating leaf gas exchange in the field to rubisco activity.

Materials and Methods

The study site is located in the Brindabella Ranges, ACT, Australia (35°23´23´´S at 148°48´13´´E). The environmental factor changing most obviously with season (beside day length) is air temperature and thus T_l. Gas exchange was measured in the field (KÜPPERS M. & al. 1987). Rubisco contents and activity were determined in the laboratory (KÜPPERS B. I. L. 1996), if possible for the same, but also on different leaves (repetitions on different leaves 2-3 per plant, two species (see below), rubisco up to 5 with 2 to 4 repetitive extractions for every leaf (KÜPPERS B. I. L. 1996) .

Results

In summer, optimum leaf temperature, T_l of A was around 23°C in a fully grown leaf (Fig. 1A). But also in early winter, after several nights with frost, optimum, T_l, was at 23°C, when intercellular CO_2 concentration, c_i, was kept similar to that in summer (Fig. 1B). The course of this experiment needs further attention (Fig. 1D, E): after a peak in A at 14°C (equivalent to maximum day temperature: arrow in Fig. 1D), A dropped at T_l of 16 and 17°C. Raising T_l further, A recovered and reached an even higher peak at 23°C. At yet higher T_l there was a sharp drop in both A and leaf conductance to water vapour, g (which declined due to a very high leaf-to-air water vapour concentration difference, Δw,



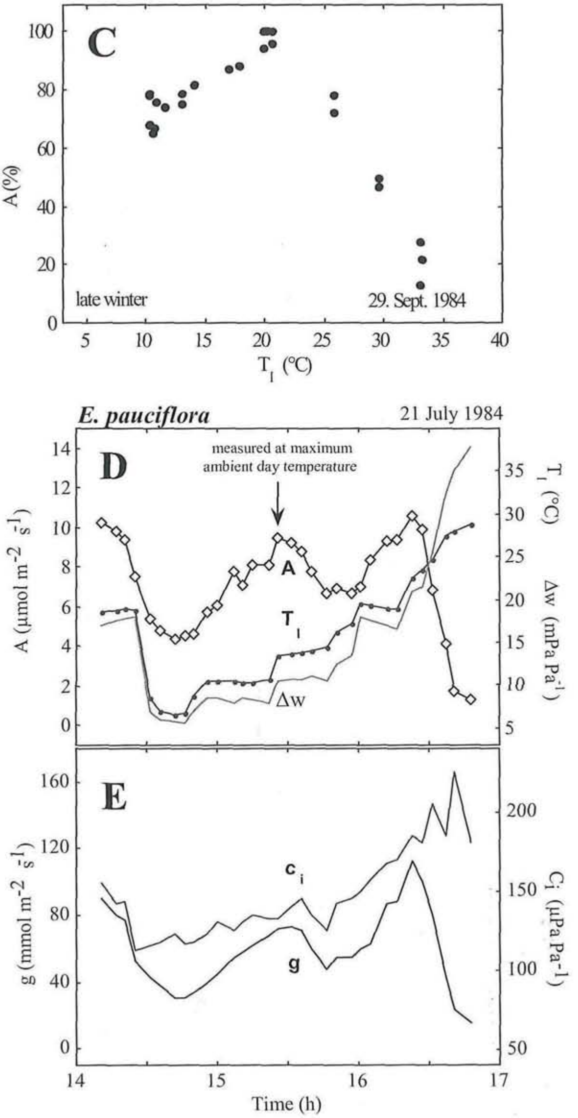
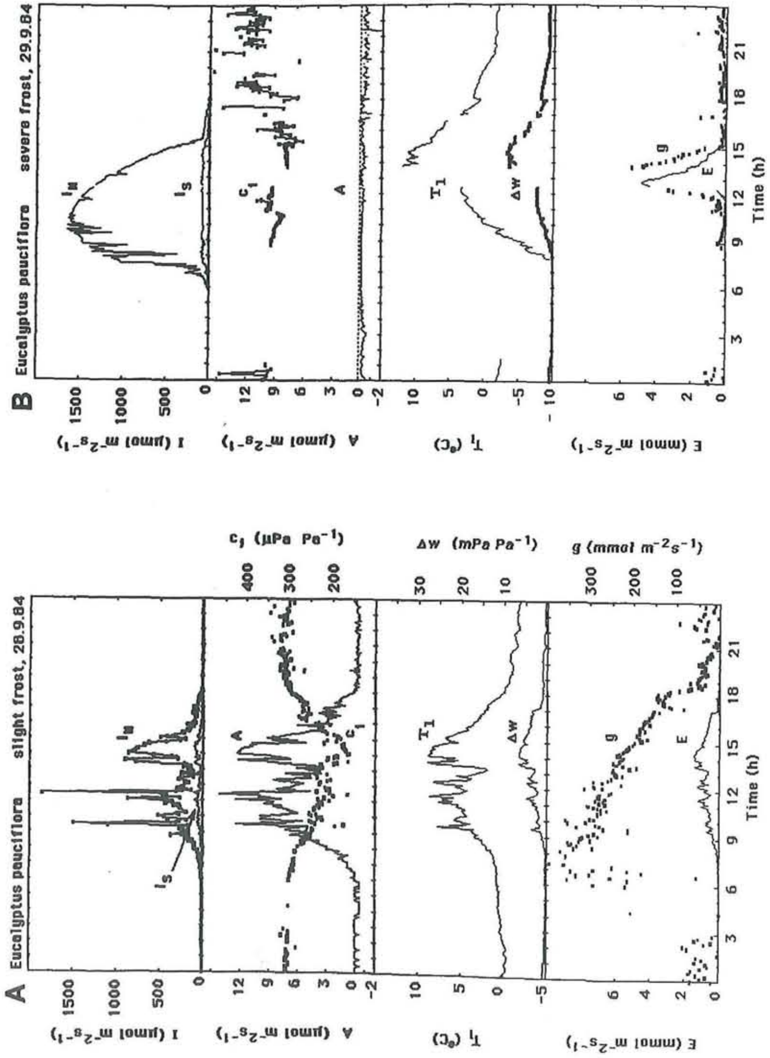


Fig. 1. Temperature responses of leaf gas exchange in leaves of *E. pauciflora* in the field. A: net photosynthesis (A) in summer, B: A in winter, C: A in late winter, D: temporal course of A, leaf-to-air water vapour conentration difference (Δw) to artificially varied leaf temperature (T_l) to yield the response curve in B, E: same as D but for intercellular CO_2 concentration (c_i) and leaf conductance (g).



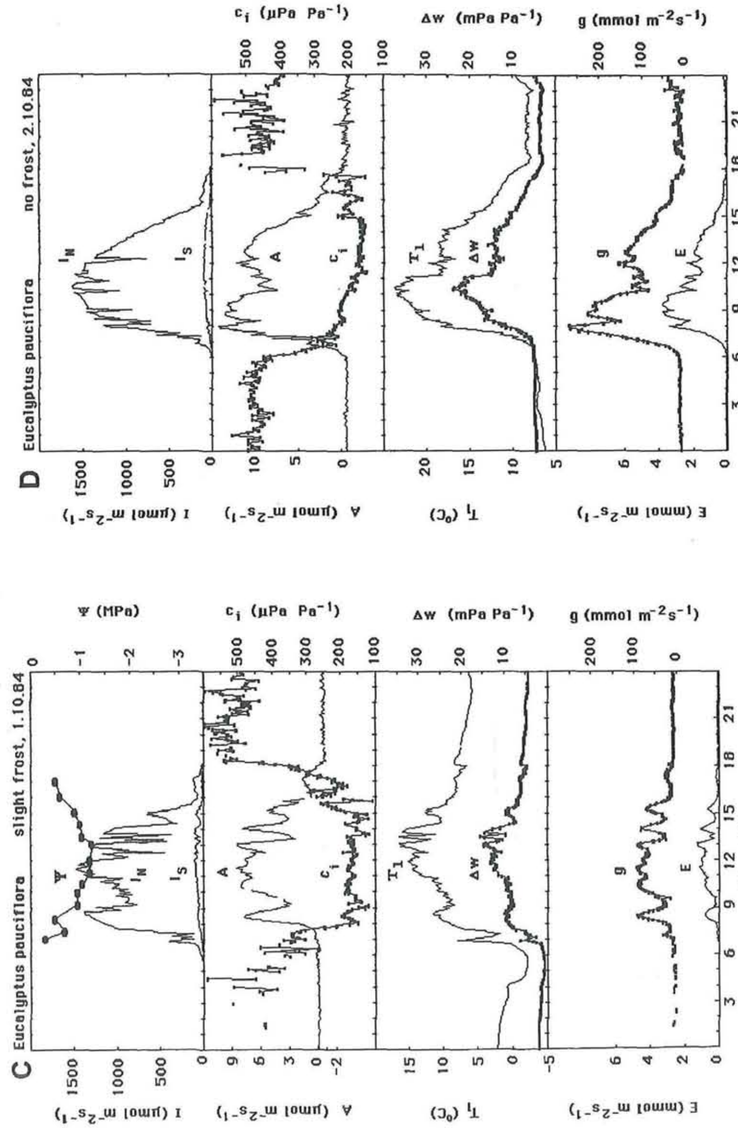
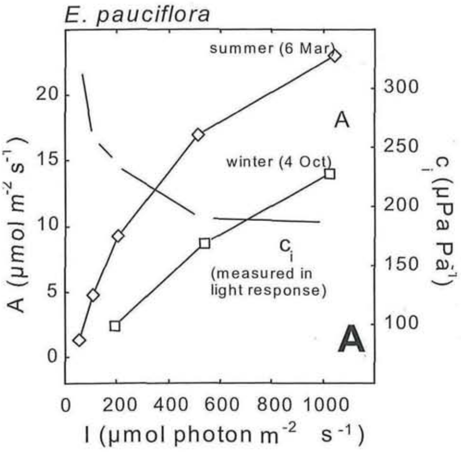


Fig. 2. Diurnal courses of leaf gas exchange in the field, all for the same leaf. A: slight frost, B: severe frost, C: slight frost after 2 days of severe frost, D: no frost. E = transpiration, I = photon irradiance, N, S = north and south facing, respectively. Other symbols as in Fig. 1.

that could not be avoided due to the low outside air temperatures during the experiment). But this was not the reason for the decline in A since c_i increased as an effect of respiration. Towards the end of winter, there was a period of regular night frosts down to -7°C lasting for several days. At that time optimum T_l for A was still 20°C (Fig. 1C). Very similar results were obtained for another eucalypt, *E. delegatensis* ("alpine ash"), at the same altitude.

Quick recovery was observed when consecutive diurnal courses of leaf gas exchange were examined (Fig. 2). The first day (28. Sept.) was a changeable one with slight frost at night (Fig. 2A). A, g and transpiration (E) showed the typical response patterns to climatic parameters (such as photon irradiance (I), T_l and Δw). In the following night, frost cooled down the leaves to at least -10°C (Fig. 2B). Thereafter, A did not respond to light at all. Whether stomata opened for a while or not and whether transpiration peaked is questionable due to the critical temperatures the measurements were performed at. The next night, T_l dropped to -5°C (not shown). Xylem pressure at predawn was highly negative (-3.1 MPa) due to frozen sections in the petioles but rapidly recovered in the sun. Despite another bright day, the leaf did not assimilate much. Stomata also showed little (but reliable) reaction during the day. Only slight frost occurred the following night (Fig. 2C). This day (1. Oct.) was slightly cloudy and photosynthesis followed the pattern of irradiance, albeit at a lower level than before the severe frost. Stomata remained fairly closed, causing a low c_i of about 160 μPa Pa^{-1} during the day. The next night was the first without frost. On the following day both A and g followed the normal diurnal course of environmental variables. Three days after the last frost (not shown), the leaf had fully recovered. Then leaf gas exchange showed no fundamental differences to diurnal courses in summer.



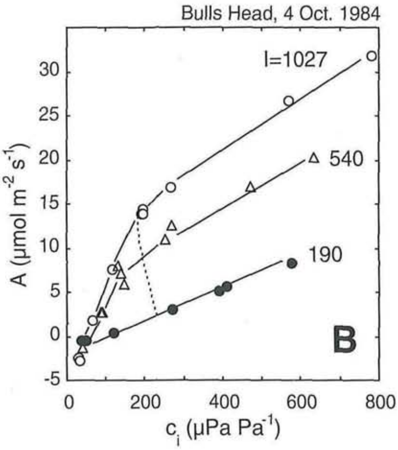
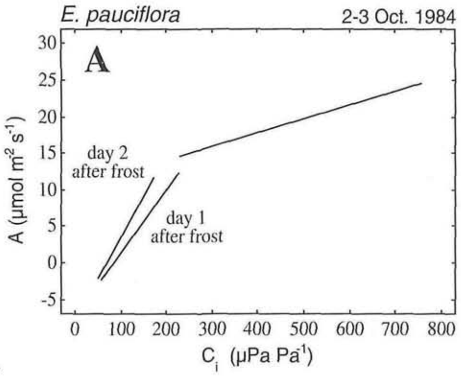


Fig. 3. A: Constructed light (I) responses of net photosynthesis (A) for a given intercellular CO_2 concentration (c_i) for a situation in summer as well as in winter. B: Example of a set of A/c_i relationships at different I to be used to construct the winter time response in A (T_1 = 22-25°C, Δw = 15-23 µPa Pa^{-1}, air pressure = 905 hPa, 21% O_2 in air).

During and shortly after frost, A was photoinhibited. This is supported by light response curves in the quantum yield region (Fig. 3). Since chlorophyll fluorescence technique was not available, the light response curves were constructed from „A/c_i curves" at different light levels (Fig. 3B). Typical (and identical) responses of c_i to light were assumed for both summer and winter (Fig. 3A). The slope of ($\Delta A/\Delta I$) at low I is clearly lower shortly after frost.



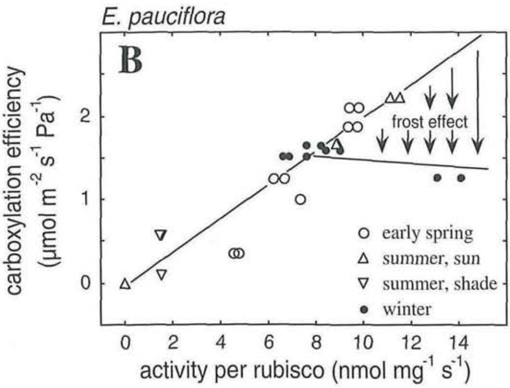


Fig. 4. A: c_i response of light saturated A 1 and 2 days after frost: recovery of $\Delta A/\Delta c_i$ at low c_i is evident (T_l = 20.5°C, Δw = 18.5µPa Pa^{-1}, air pressure = 905, 21% O_2 in air). B: Carboxylation efficiency ($\Delta A/\Delta c_i$ at low c_i), measured in the field at conditions of A, as related to potential activity per rubisco of the same leaf, respectively.

Frost also reduced photosynthetic capacity (A_{max}, not shown) and the so-called "carboxylation efficiency", $\Delta A/\Delta c_i$, at low c_i (Fig. 4A). However, rubisco content (not shown) and potential rubisco activity in the same leaves at the same time were not affected (Fig. 4B).

Discussion

The small shift in optimum T_l of A (3 to 5°C) contrasts other observations, where a shift of more than 10°C in the course of a year was measured (SLATYER & MORROW 1977). Certainly, stomatal effects play an important role: due to very low dew points in winter (especially after frost), Δw at higher T_l during artificial temperature response curves could have been much higher than in summer. Thus, with increasing T_l, g was successively reduced and by that c_i (here particularly between 15:30 and 16:00 (Fig. 1D) resulting in a pseudo-temperature-optimum of A at 13°C, Fig. 1B). Most likely, in the experiments of SLATYER & MORROW 1977 c_i was not kept (more or less) constant as during the present experiment. Thus, their results cannot be directly compared. Ours allow for an interpretation on a more physiological basis. However, the results of SLATYER & MORROW (see OSMOND & al. 1987) present an idea of the actual carbon gain at a given T_l (and favourable I). Our observations agree with those of KÖRNER & DIEMER 1987 who found identical physiological T_l responses of A in *Ranunculi* from either the Inn valley near Innsbruck (ca. 600m a.s.l.) or at the Patscherkofel (about 1900m a.s.l.).

Basically, A responses in winter were the same as in summer. Clear differences only came up after severe frost. At T_1 below -5°C the photosynthetic apparatus was affected (lower initial slope in A/c_i curves in Fig. 4A, with no photosynthesis during diurnal courses, see Fig. 2B), but leaves fully recovered after several days without frosts since potential rubisco activity was fully maintained during frost (Fig. 4B). In *Abies alba* , PISEK & KEMNITZER 1968 observed a net CO_2 release after frost with a slow, but not always complete, recovery. BALL & al. 1991 found differences in photosynthetic light response of leaves in winter. They measured that leaves of *E. pauciflora* exposed to high light after frost showed reduced quantum yield whereas covered leaves obtained values similar to those in autumn.

Obviously, the situation of *E. pauciflora* as well as of *E. delegatensis* (KÜPPERS B. I. L. 1992) differs from that of the situation in the European Alps, since there LARCHER & BAUER 1981 (see also SAKAI & LARCHER 1987, there p. 238) observed that full recovery of the photosynthetic capacity after winter stress requires weeks. However, in their cases frosts were long lasting and certainly affected soil temperature and soil conditions much more than in our study.

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