

**THE ECOLOGICAL SIGNIFICANCE OF LEAF MOVEMENTS  
IN RHODODENDRON MAXIMUM**

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(ABSTRACT)

Although leaf movements have been documented for over a century, there are few studies focused on the adaptive significance of leaf movements, especially with experiments under controlled conditions. The major objective in this study is to determine the ecological significance of leaf movements in *Rhododendron maximum*, which is a subcanopy, evergreen species distributed in seasonally cold environments. Leaf movements could be necessary for maintaining a favorable energy balance and/or avoiding photoinhibition and photooxidation. A series of leaf manipulation treatments were established to verify these potential explanations. These leaf treatments were designed to separate the influence of leaf curling and leaf angle on leaf energy budget, gas-exchange characteristics, chlorophyll contents and leaf longevity.

Leaf movements were found to have a significant influence on leaf physiology and longevity of *R. maximum*. Without changes in leaf angle, chlorophyll contents decreased, and permanent photoinhibition occurred due to excess

irradiance absorption in the winter. Leaf angle also influenced leaf temperature although the changes in leaf temperature were within the physiological tolerances of *R. maximum* leaves. Leaf curing had little or no effect on the parameters measured in the study.

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# Introduction

Leaf movements have been documented for over a century (Darwin 1885). Following the early work of Darwin, environmental stimuli for leaf movements have been extensively studied. Leaf movements can be classified into several groups according to the major effective environmental factor. For instance, nyctitropic leaves respond to darkness, and heliotropic movements are determined by the direction of light. Leaves of diaheliotropic plants, solar tracking plants, track the movement of the sun to receive as much solar radiation as possible. On the other hand, leaves of paraheliotropic plants move to minimize the absorption of light during the middle of the day. Leaf movements are also caused by temperature (thermotropic) and moisture (hygroscopic).

Although many patterns of leaf movement have been well documented, the ecological significance of leaf movement has been studied in only a few cases. For



example, heliotropism and paraheliotropism, usually found in high irradiance environments, relate to energy balance and photosynthesis ( Forseth and Ehleringer 1983 ). On the other hand, nyctitropic and thigmotropic movements may conserve leaf nutrients ( Wallace, Timpano and Durgin 1987 ) and reduce herbivory. Thermotropism is one of the least understood patterns of leaf movement, although it has been suggested that these movements may prevent leaf freezing and/or leaf desiccation during winter months ( Fukuda 1933; Havis 1964 ).

*Rhododendron* species serve as one of the best groups for studying thermotropic leaf movements. Leaf movement in *Rhododendron* species occurs throughout the distribution of *Rhododendron*, and it has been measured in the southwestern and eastern U.S.A.(Nilsen 1985; Havis 1964 ) and in Asia (Fukuda 1933). Recent studies (Nilsen 1985) reveal that leaf curling and changes in leaf angle are related to several external and internal factors. For instance, leaves of *R. maximum* start curling and drooping when leaf temperature is below -1 C. Experiments under field and lab conditions also show that as the leaf water potential decreases, the leaf curling and drooping increases (Nilsen 1987). In addition, field evidence indicated that leaf angle is related to irradiance.

Several hypotheses have been suggested to explain the significance of leaf movements in *Rhododendron*. First, leaf curling may serve to avoid drought resulting from low temperature in the winter. High leaf water deficit in late winter, when the soil in the root zone is frozen, may cause desiccation injury to

*R. catawbiense* leaves (Havis 1964). Leaf curling may prevent water loss during the winter by creating a moist microclimate around the stomata or preventing the stomata from opening (Fukuda 1933; Havis 1964). This interpretation is confounded because leaf temperature and leaf water potential are correlated (Nilsen 1987). In addition, temperature controlled laboratory experiments reveal that the decrease of leaf water potential is only partially responsible for leaf curling ( Nilsen 1987 ).

Leaf orientation of *Rhododendron* could also be important to the energy environment of the leaves ( Gates 1980; Mooney et al 1977; McMillian and McLendon 1979; Geller and Smith 1982; Forseth and Ehleringer 1982). Leaf temperature can become as much as 15 C above air temperature ( Gates 1963; Regehr and Bazzaz 1976) or 15 C below air temperature (Smith 1978) depending on environmental and leaf conditions. Leaf energy-exchange characteristics may be important determinants of plant physiological functions or even survival ( Gates 1968; Baskin and Baskin 1978 ). For example, a horizontal *R. maximum* leaf at 0 C air temperature and -23 C sky temperature has a leaf temperature of -6 C ( the leaf freezing point); while a vertical leaf under the same conditions has a leaf temperature similar to air temperature (Nilsen unpublished data). Studies on the interaction of leaf orientation and leaf seasonal or temporal energy budget have been carried out in many desert plants (Badger et al, 1984; Mooney and Ehleringer 1978), but not in temperate subcanopy plants, such as *Rhododendron*, in the winter.

Third, leaf movements may protect leaves from photoinhibition and photooxidation particularly under conditions of high irradiance and cold temperature. High light intensities may damage the photosynthetic apparatus, or cause photoinhibition of shade plants because of the balance between the quantity of light absorbed and the photochemical capacity of plants ( Long, East and Baker 1983 ). Experiments have also shown that plants are more sensitive to light intensity at low temperature ( Powles 1984; Strand and Oquist 1985; Ogren and Oquist 1984 ).

There are few studies focused on the adaptive significance of leaf movements of a sub-canopy species in a seasonally cold environment. Furthermore, to our knowledge, these questions have not been investigated experimentally under controlled conditions. As a result, the basic ecophysiological data necessary to evaluate the significance of thermotropic leaf movements in *Rhododendron* are not available.

My objective in this study is to determine the ecophysiological significance of leaf movements in *R. maximum*. Does the significance of leaf movements relate primarily to energy balance, or are other factors such as desiccation, photoinhibition, photooxidation and water-use efficiency involved? We began our study with the following possible explanations: 1) leaf movements are necessary for a favorable energy balance of plants. Thus, without leaf movement, leaf temperature would drop below air temperature during cold nights, or leaves would overheat during a clear midday in winter. 2) Leaf movements are

necessary to avoid photoinhibition of the electron transport system in chloroplasts during the winter when irradiance is high and leaf temperature is low. 3) Leaf movements are necessary to avoid the permanent photooxidation of chlorophyll. The approach we used to evaluate these possibilities was to establish a series of leaf manipulation treatments, which forced leaves to maintain specific orientations. We investigated these possible explanations by determining the influence of leaf treatments on leaf longevity, energy budget, gas-exchange characteristics and chlorophyll contents.

# Materials and Methods

## Species and Site Description

*R. maximum* is a subcanopy, evergreen shrub, which is distributed in the Appalachian mountain region from Georgia into Canada; however, *R. maximum* populations reach their greatest dominance in the Appalachian Mountains of Virginia and West Virginia, U.S.A.. Leaf size of *R. maximum* ranges from 20 to 200  $cm^2$  with an average of 80  $cm^2$  for mature leaves. Six to ten annual leaves are located in a tight leaf whorl with a long internodal section of stem. New leaf expansion and shoot elongation occur over a short period of time, 1-2 weeks, in the middle of May ( Nilsen 1985 ), so that leaf age within a cohort of whorls is uniform. *R. maximum* leaves survive from 3 to 7 years depending on the microclimate ( Nilsen 1986 ). The ease in determining leaf age makes it possible

to establish treatments within an even aged cohort, thereby eliminating leaf age effect. Leaves are thick making them resistant to mechanical and insect damage.

The research site was located in a valley of the Jefferson National Forest nearby Blacksburg, Virginia, U.S.A. ( Longitude =  $80^{\circ}22'59''$ ; Latitude =  $37^{\circ}15'47''$ ), and had a slope of 0 degrees and an elevation of 640m. The canopy was dominated by deciduous species ( *Quercus alba* and *Acer rubrum*). *R. maximum* was the dominant subcanopy species. A more complete vegetation and site description was available elsewhere ( Nilsen 1985 ).

## Leaf Manipulation

In August, 1985, six different leaf treatments were established in the natural population to examine the effects of leaf curling and leaf angle on leaf energy budgets and photosynthesis. They were the following:

1. *CONT group*; control leaves, the leaf curling and angle are not restrained.
2. *VC group*; the leaves are restrained in a vertical position, but they are allowed to curl.
3. *HC group*; the leaves are restrained in a horizontal position, but they are allowed to curl.

4. *FF group*; the leaf angles are free to change, but the leaves are not allowed to curl.

5. *VF group*; the leaf angles are restrained in a vertical position, and the leaves are not allowed to curl.

6. *HF group*; the leaf angles are restrained in a horizontal position, and the leaves are not allowed to curl.

In order to reveal the treatment effects, several precautions were taken. Only leaves of the same age and free from any obvious insect damage were used. Each treatment included 175 leaves, among them 100 leaves were 1 year old and 75 were two years old. The six treatments totaled 1050 experimental leaves. Sample leaves were selected from healthy appearing branches in the canopy of three *R. maximum* individuals growing near each other.

On each leaf two holes were punched in the middle of the leaf blade on each side of the main vein. The holes were used to support a frame made of thin, insulated wire and a piece of thread. The wire frame is used to prevent the leaf from curling; and the thread is used to restrain the leaf at a specific angle. On each control leaf two holes were punched and a frame was attached without restricting leaf movement. Preliminary studies showed that there were no significant effects of punching and frame attachment on leaf photosynthetic characteristics. The six different treatments were assigned respectively to six leaves in each even aged

whorl on each branch to enable a determination of the variance resulting from the influence of branches.

## **Measurements**

Just after the leaf treatments established, on September 25, 1985, a diurnal cycle of leaf temperature, stomatal conductance, and light intensity was measured with a LI-COR Model LI-1600 steady state porometer. Air and soil temperatures were measured with thermocouples (copper -constantan; 36 gauge); and leaf water potential was determined with a pressure chamber (PMS instruments, Model 1000).

Observations of visible changes in sample leaves, such as chlorosis or abscission, were made during the entire experimental period. These data were collected to determine the influence of the treatments on leaf survivorship.

Diel cycles of sample leaves and air temperatures were measured with thermocouples and recorded with a micrologger, (Campbell; Model CR21) to determine the consequence of leaf movements on the leaf energy budget. Thermocouples were embedded into the lower surface of the leaf. Temperature data were collected each five minutes from Feb. 10 to Feb. 17, 1986 and April 10 to April 20, 1986 on two sets of the six treatments.



Leaf chlorophyll ( chl) content was determined with UV-VIS spectrophotometer (Gilford response) on January 14, Feb. 25, March 27 and May 15, 1986. Each time 16 branches were collected, so that each treatment had 16 sample leaves. Ten leaf disks were punched from each leaf. The chlorophyll was extracted by grinding leaf disks of known area in 80% acetone. The homogenates were centrifuged at 100g for 5 min. Absorptances of the supernate were measured at 664 and 647 nm. From these absorptances, chl.a, chl.b, total chl (chl.a + chl.b) and chl.a/b were calculated.

Gas-exchange measurements were conducted with a null-balance, gas-exchange system (Data Design Group, Model PACsys 9900) ( Field, Berry and Mooney 1982 ), in the spring and summer of 1986. This is a complete, computer-based portable system for making automated gas exchange measurements on plant leaves in both the field and lab. The system utilizes two microcomputers for control and display applications. The control/data aquisition computer is housed in the main system unit, together with the intergral data aquisition system, mass flow controller (Tylan Corporation), and infrared gas analyzer (Liston Edwards Incorporated). The control system communicates with the console computer ( Radio Shack Model 100; Tandy Corp). The console accepts data from the control computer, processes the basic data, and displays the data in both tabular and graphical form on the console screen. During the measurements, a single attached leaf was inserted into the cuvette. The temperature, moisture and  $CO_2$  content were controlled in the cuvette. A mass balance approach was utilized in budgeting fluxes into and out of the leaf cuvette. Gases from three cylinders

containing 3000ppm  $CO_2$ , 320 ppm  $CO_2$  and pure  $N_2$  ( $CO_2$  free air ) respectively were used to regulate the humidity and  $CO_2$  level in the cuvette. The fluxes were determined using a steady-state "null balance" approach in which dry air and carbon dioxide were precisely metered into the cuvette at rates which just compensate transpiration and net carbon dioxide exchange, thus maintaining constant cuvette humidity and carbon dioxide concentration. Leaf temperature was measured with a 36 gauge copper-constantan thermocouple inserted into the leaf lower surface. Leaf temperature was automatically controlled inside the cuvette. Light was provided by a sylvania capsylite flood lamp with an appropriate housing, heat filter and neutral density filters. The light intensities were controlled by changing the number of neutral density filters. Light intensities on the leaves were measured with a quantum sensor (Licor, Model 190s). Photosynthesis, leaf conductance, transpiration and internal  $CO_2$  concentration were calculated ( Field et al 1982 ).

Curves of photosynthetic response to temperature were obtained by starting photosynthetic measurements at a leaf temperature of 25°C, then decreasing the leaf temperature in steps to 18°C. The temperature was then increased in steps (5-7 °C) until 42°C was reached. The optimal temperature obtained was used in all light response measurements.

Similarly, in the photosynthetic-light responses, leaves were first exposed to light at a intensity of 270  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (400-700nm). After a constant photosynthetic rate had been obtained, the light was lowered to total darkness then increased in

steps to of 20-50  $\mu\text{mol m}^{-2} \text{s}^{-1}$  to 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  (400-700 nm ), at each step a constant photosynthetic rate was attained before advancing to the next higher light level. Leaf temperature was maintained at the optimal condition, 30°C, and vapor pressure deficit (VPD) levels were kept at less than 1.0 KPa. Ambient ( air in cuvette)  $\text{CO}_2$  levels were maintained at 350  $\mu\text{mol mol}^{-1}$ .

## Results

The results of measurements conducted on Sept. 25, 1985, just after leaf treatments were established, showed that there was no significant difference ( t-test, 95% confidence level , SAS, 1982 ) in leaf conductance and leaf transpiration among treatments. Leaf conductance and transpiration of both control and treated groups were lower in the early morning, reached their peak at noon, then decreased slowly ( Figs. 1 and 2 ). There was also no significant difference ( t-test, 95% confidence level ) in leaf water potential among the treatment groups. Leaf water potential was high ( less negative ) in the early morning, reached the lowest value at 14:00 h, then increased again ( Fig. 3 ). Light intensities on the surface of leaves were different among the treatments, i.e. horizontal groups > control > vertical ones at mid-day ( Fig. 4 ). Irradiance levels were low in comparison to winter irradiance (  $90 \text{ } \mu\text{mol } m^{-2} s^{-1}$  for horizontal groups ), because of light interference by the canopy of deciduous

trees. Leaf temperatures among the treatments were not significantly different ( difference less than  $1^{\circ}\text{C}$  ).

During the winter and early spring, before the deciduous canopy trees produced leaves, the light environment was quite different among the treatments. For example, measurements made at mid-day of March 30, 1984 (Nilsen, personal communication ) revealed that the light intensity on horizontal leaves (  $130 \text{ umol } m^{-2} s^{-1}$  ) was significantly ( t-test, 95% confidence level ) higher than that of more vertical leaves (  $90 \text{ umol } m^{-2} s^{-1}$  ).

Diel temperature patterns were variable among the days measured, therefore, we have selected one diel cycle to represent the range of characteristics observed ( 16 days ). In the early spring (April, 10 ), before leaf emergence, there was a wide range of air temperatures from  $1^{\circ}\text{C}$  at 0600 h to  $23^{\circ}\text{C}$  at mid-day ( Fig. 5 ). The average leaf temperature was higher than the air temperature during the day from 8:00 to about 15:00 and the largest difference between the leaf and air temperature (  $4^{\circ}$  to  $8^{\circ}\text{C}$  ) was found at mid-day ( Fig. 6 ). Leaf temperatures were lower than air temperatures during the other hours of the day (1500 h - 08:00 h ), but the difference was less than  $1^{\circ}\text{C}$  for all treated groups ( Fig. 6 ).

The comparison of leaf temperatures among different groups revealed that the horizontal treatment had leaf temperatures higher than that of vertical ones during the day ( Fig. 7 ). The vertical leaf temperature was close to air temperature during the entire day (difference less than  $1^{\circ}\text{C}$ ) ( Fig. 7 ). On the

other hand, the leaf temperature of horizontal groups was lower than that of vertical ones at night, and above air or vertical and control leaf temperature ( up to 7 °C ) during the day ( Fig. 7 ).

During mid-day ( from 1200 h to 1500 h ), the changes of vertical leaf temperature were less than 2.5 °C on a short term basis ( five minute interval ). However, there were sharp temperature changes in horizontal leaves ( Fig. 8 and 9 ). Leaf temperature could change 5 °C or greater in 5 minutes. The steep leaf temperature increases were due to sunflecks. This result indicated that the light environment was quite different between the horizontal and vertical leaves. That is horizontal leaves received more sunflecks than vertical ones. On the other hand, the different patterns of temperature change between the *CH* ( curling free and restrained horizontal ) and *FH* ( restrained flat and horizontal ) groups were due to the position of individual leaves within the variable pattern of sunflecks in the subcanopy.

Temperature curves of photosynthesis were carried out to determine the optimal temperature for photosynthesis of *Rhododendron maximum*. The results of the photosynthetic temperature responses showed that the optimal temperature was 30 °C ( Fig. 10 ). Rates of net photosynthesis rose steeply below the temperature optimum and declined more slowly above the optimal temperature. During the summer, average maximum air temperature in the habitat was 23 °C. It was interesting to note that *R. maximum* had a temperature optimum for

photosynthesis  $7^{\circ}\text{C}$  higher than the air temperature. However, the temperatures of horizontal leaves in sunflecks could rise  $5^{\circ}$  or  $6^{\circ}\text{C}$  above air temperatures.

The measurements of light dependence of photosynthesis were carried out in July and August of 1986 in order to detecting permanent photoinhibition.

Photosynthesis, as a function of light intensity at optimal temperature and ambient  $\text{CO}_2$  concentration, showed a linear response to photon flux density up to  $160 \text{ umol } m^{-2} s^{-1}$ . Net photosynthesis saturated at photon flux density near  $250 \text{ umol } m^{-2} s^{-1}$  ( Fig. 11 ). However, there were substantial differences in light response curves among the groups. First, the average maximum photosynthesis rate of control leaves was significantly ( t-test, 95 % confidence level ) higher than that of other groups ( Table 1 ). The maximum photosynthetic rates of other treated groups ranged from  $1.03$  to  $1.32 \text{ umol } m^{-2} s^{-1}$  which were not significantly different at the 95% confidence level (t-test ) ( Table 1 ). Secondly, the net photosynthesis of *FF* group, vertical and horizontal leaves was lower than that of control leaves at lower irradiance levels (  $< 150 \text{ umol } m^{-2} s^{-1}$  ) ( Fig. 12 ). For example, the photosynthesis of vertical and horizontal leaves was 49.5 % and 58.6 %, respectively, compared to control leaves at light intensity of  $100 \text{ umol } m^{-2} s^{-1}$ . The incident quantum yield of control leaves (  $.0095 \text{ mol } \text{CO}_2/\text{mol}$  quanta ) was also higher than that of vertical (  $.0065 \text{ mol } \text{CO}_2/\text{mol}$  quanta ) and horizontal leaves (  $.0063 \text{ mol } \text{CO}_2/\text{mol}$  quanta ).

Chlorophyll contents were analysed to determine levels of photooxidation.

Previous studies showed that there were no significant differences of chlorophyll

content among the same age leaves prior to treatment ( Nilsen and Bao unpublished data ). Chlorophyll concentrations of all six treatments decreased with increasing leaf age. However, the percentage decrease was different among the groups. The horizontal groups decreased more than other treatments ( Fig. 13 ). Measurements conducted on Jan. 12, 1986 revealed that there was no significant difference of total chlorophyll content among the six treatments. Four months later ( May measurement ), total chlorophyll content differences could be recognized at the 95% confidence level ( Duncan's test, SAS, 1982 ), where chlorophyll content of horizontal leaves was lower than other groups ( Table 2 ). Because the chl<sub>a</sub>/chl<sub>b</sub> ratio was stable during the measurements, only total chlorophyll ( chl<sub>a</sub> + chl<sub>b</sub> ) was presented.

There were no significant differences in leaf water conductance, transpiration, intercellular  $CO_2$  concentration and water use efficiency between the treatments ( t-test, 95% confidence level ) ( Table 2 ). There was also no visible evidence, by examining the electro-micrograph, to indicate that treatments affected the substructure of chloroplasts. The percentage of leaf survivorship of each treatment was calculated on April 10, 1987. The survivorship of control group was similar to that of untreated leaves ( Nilsen, 1986 ), 96% for age 2 leaves and 54 % for age 3 leaves. The survivorship of other treatments was lower than that of control leaves ( Fig. 14 ). The difference between horizontal and vertical leaves was greater than that between the curling free and restrained no curling groups. The lowest survivorship was found in vertical leaves.



## Discussion

The primary objective of this study was to determine the ecological significance of leaf movements. The approach included an examination of the energy budget and some physiological characteristics of leaves. Leaf angle and aspect are related to energy balance and may have adaptive importance in some desert plants ( Mcmillen and McClendon 1979; Mooney, Ehleringer and Bjorkman 1977; Forseth and Ehleringer 1982 ). The leaves of *R. maximum* are large, entire and thick, consequently, they have a thick boundary layer ( Parkhurst et al 1968 ). Also, during the winter and early spring, transpiration is inhibited because stomata are closed, therefore, latent heat exchange is minimal ( Nobel 1983 ). Horizontal leaves, exposed to a relatively open sky during the winter under a deciduous canopy, may lose more infrared energy at night and receive more total energy at mid-day than vertical leaves. The horizontal leaves cannot effectively release the absorbed energy through conduction, convection, or latent heat

exchange. Consequently, the temperature of horizontal leaves is likely to raise above air temperature during the day and drop below air temperature at night. I found that the temperatures of horizontal leaves could be lower than control leaf and air temperatures by 1 °C at night and 6° or 7 °C over air temperature during the day ( Fig. 6 ).

The adverse effects of low ( chilling or freezing ) temperatures on plants have been reported in many species ( Powles, 1984 ). However, these effects are reduced in darkness even for cold-sensitive plants ( Lasley, Garber and Hodges 1979; Van Hasselt and Van Berlo 1980; Powles et al 1983 ). *R. maximum* is a low temperature tolerant species, in which leaves withstand temperatures ( -15 °C to 20 °C ) below freezing point ( -7 °C ) without any damage during the winter.

Therefore, the difference in temperature ( < 1 °C ) between the horizontal and control leaves is not likely to be physiological critical. Additionally, previous studies ( Nilsen, 1986 ) demonstrated that leaf drooping occurs at -1 °C, which is 5 °C higher than the freezing point of *R. maximum* leaves. Based on the above observation, I feel that the leaf drooping movements of *R. maximum* play only a minor role in avoiding potential freezing damage by ameliorating the infrared radiation balance.

On the other hand, it has been shown that the rate of temperature change is a critical factor associated with freezing damage ( Powles, 1984 ). The results of this study reveal that leaf temperatures could increase 5° to 7 °C in a short period of time ( within 5 minutes ) when leaves are exposed to sunflecks. Leaf curling

could reduce the speed of leaf thaw by reducing the irradiance load, thereby avoiding potential damage by rapid thaw.

Sunflecks play an important role in both energy balance and photosynthesis for subcanopy species ( Chazdon and Pearcy, 1986 ). In our study, during the winter, the horizontal leaves of *R. maximum* receive more sunflecks than the vertical leaves, consequently, the temperatures of horizontal leaves could be 7° to 8° C over air temperatures. Overheating can induce leaf damage ( membrane leakage ) in many species ( Monson et al, 1982 ). However, it seems unlikely that the change in leaf angle or curling is a mechanism to avoiding thermal damage, because leaves are at more or less horizontal position when leaf temperatures are above 0° C. In addition, the thermal response of photosynthesis had an optimal temperature of 30° C, which was 7° C above the average maximum air temperature in the summer.

Irradiance was often a limiting factor for many subcanopy species. Light intensities under the canopy were usually lower than  $100 \text{ umol m}^{-2} \text{ s}^{-1}$ , which was below the light saturation point ( $250 \text{ umol m}^{-2} \text{ s}^{-1}$ ) of *R. maximum*. Consequently, photosynthetic rates were low. If the leaves had an optimal temperature for photosynthesis of 23 °C ( the average air temperature ), when leaves were exposed to sunflecks, the benefit of the increased light intensities for photosynthesis would be counteracted by the negative influence of supraoptimal temperature. In this situation sunflecks could not be utilized efficiently. The fact that the thermal optimum for photosynthesis is higher than the average

maximum air temperature also indicates the potential importance of sunflecks to the carbon balance of *R. maximum* leaves.

Examining the effects of treatments on photosynthetic characteristics provided an alternative way to determine the ecological significances of leaf movements. Photoinhibition induced by illumination of plants at low temperature can be due to the inhibition of electron transport, photophosphorylation and enzyme activity ( Powles, 1984 ). Photoinhibition is frequently determined by either chlorophyll fluorescence or gas exchange measurements. Gas exchange measurements were conducted after the leaf treatments are established for one year, in order to detect permanent photoinhibition. Fluorescence techniques are frequently used to evaluate temporary photoinhibition. Photoinhibition could be manifested as either a reduction in quantum yield or a reduction in maximum photosynthetic capacity. The marked reduction of maximum photosynthesis of horizontal leaves compared to control leaves ( Table 1 ) indicates the occurrence of photoinhibition, which may be due to the reduction of enzyme activity. The lower quantum yield of treated leaves seems to suggest that the inhibition of the light reactions may be also involved. It has been reported that shade plants have much lower electron transport capacities than sun plants ( Bjorkman at al 1972b; Boardaman at al 1972 ). *R. maximum* is a shade plant, therefore, photoinhibition could be induced by the absorption of excess excitation energy at the photosynthetic reaction center in the winter ( higher light environment and low temperatures ). Low intercellular oxygen partial pressure, because of the closed stomata, would increase this adverse effect.

Stomatal conductance of both control and treated leaves decreased proportionally to photosynthesis and light intensity, consequently, intercellular CO<sub>2</sub> pressure was constant. It seems that the difference in quantum yield among the treatments is not due to stomatal effects. Bjorkman et al ( 1972a ) points out that stomatal conductance imposes only a small limitation on photosynthesis in shade plants, although stomatal conductance does respond to variation in light intensity in shade plants.

Membrane integrity could be disrupted by the low temperature and high irradiance ( Berry and Bjorkman 1980 ). However, there was no visible evidence in this study to indicate structural membrane damage of chloroplasts as seen in electronmicrographs. The photoinhibition at this stage may not be caused by altered chloroplast structure.

Previous studies ( Nilsen and Bao, unpublished data ) reveal that chlorophyll contents of *R. maximum* leaves decrease with leaf age. The chlorophyll analysis in this study is consistent with that result. However, horizontal leaves absorb more irradiance than vertical and control leaves during the winter, which could accelerate the oxidation of chlorophyll. The disproportionate decrease of chlorophyll content in horizontal leaves, compared with the control leaves ( Fig. 13 ), could be the result of photooxidation.

Horizontal leaf longevity decreased by 30 % and photosynthesis was reduced 50 % at 100  $\mu\text{mol m}^{-2} \text{s}^{-1}$  ( the normal light intensity during the growth season ),

compared to control leaves. As a result, the productivity of the horizontal leaf cohort was reduced 65 %.

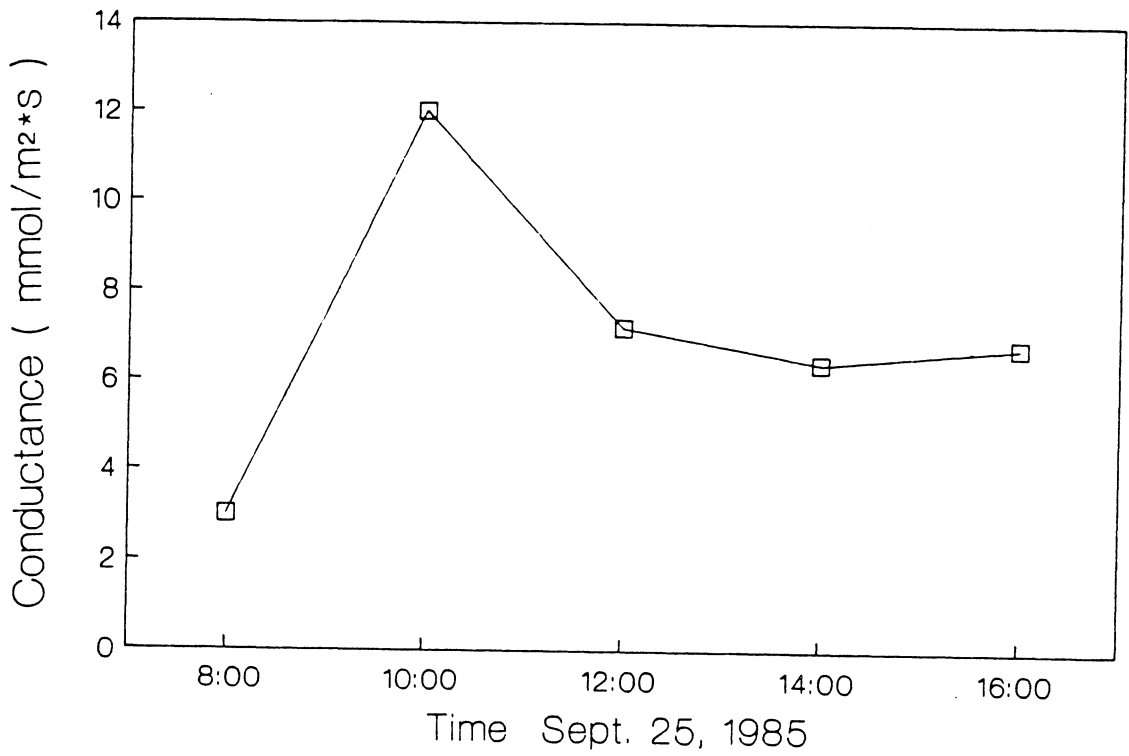
In conclusion, leaf movements were found to have a significant influence on leaf physiology and survivorship of *R. maximum*. Without changes in leaf angle, chlorophyll contents decrease, and permanent photoinhibition occurs due to excess irradiance absorption in the winter. Leaf angle also influenced leaf temperature although the changes in leaf temperature were within the physiological tolerances of *R. maximum* leaves. Leaf curing had little or no effect on the parameters measured in this study.

There are several ways in which improvements could be made in this type of study. The orientation could be enforced only during the winter and released during summer months when leaf movement is normally not occurring. For example, the low photosynthesis of vertical leaves may be induced by strain and related physiological factors during the spring and summer rather than photoinhibition during the winter.

Several other research projects could be carried out to clarify the ecological significance of leaf movements. 1.  $CO_2$  dependence curves could be conducted to further separate the influence of stomatal conductance and enzyme activity on photosynthesis. 2. Alternative techniques ( fluorescence ) of measuring photoinhibition could be used to identify the specific changes in light reaction components. 3. A more comprehensive productivity analysis at the leaf

population level could be conducted to establish a carbon gain model which may provide more evidence for the ecological significance of leaf movements in *R.*

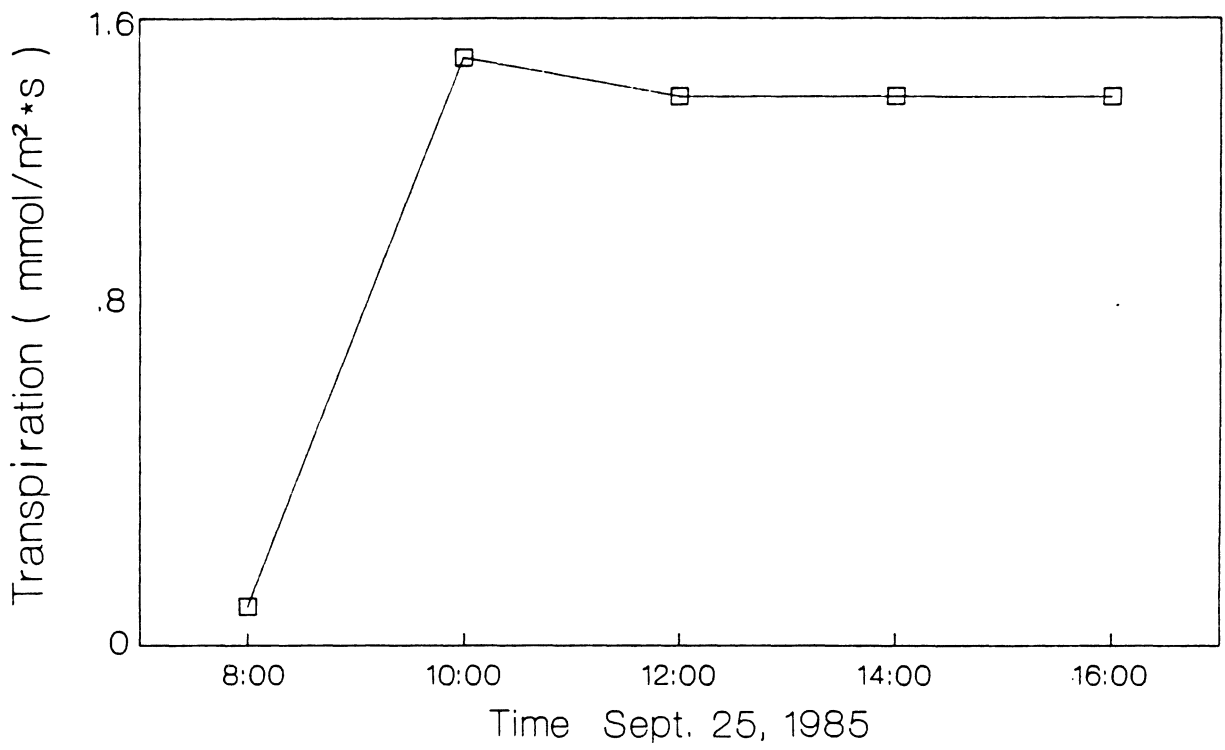
*maximum.*



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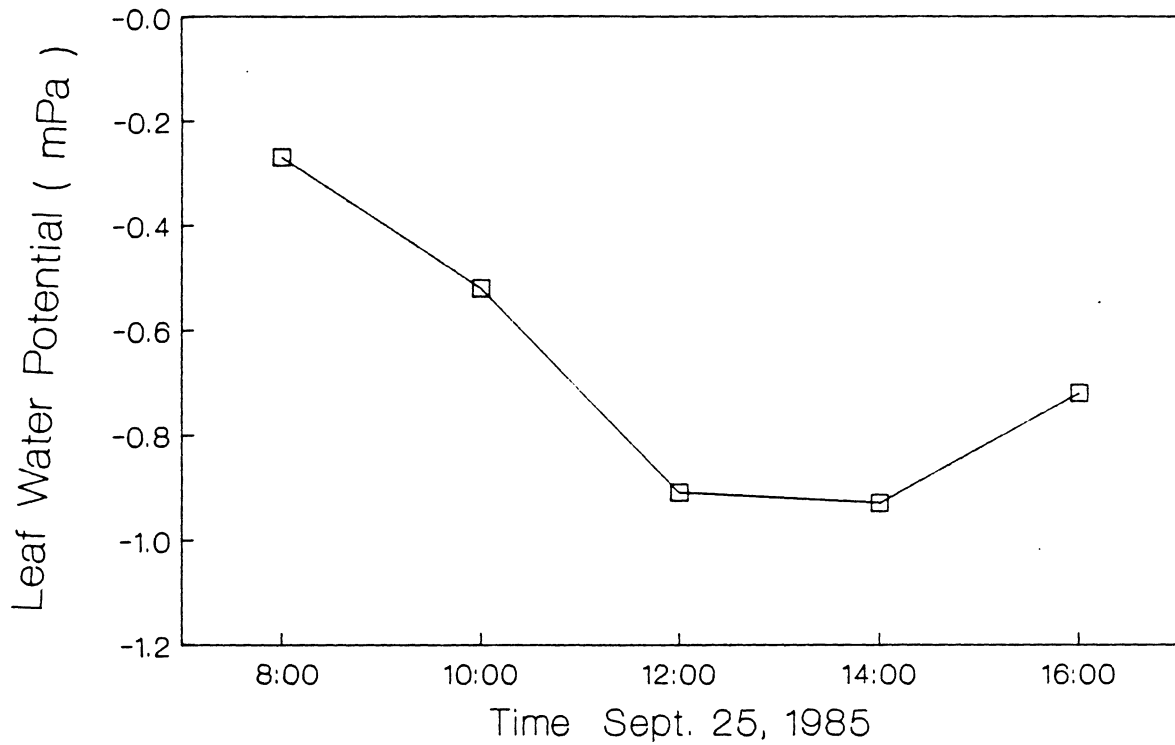
**Figure 1.** Diurnal course of the average conductance in six treatments: Measurements were made on September 25, 1985





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Figure 2. Diurnal course of the average transpiration in six treatments: Measurements were made on September 25, 1985



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Figure 3. Diurnal course of the average leaf water potential in six treatments: Measurements were made on September 25, 1985

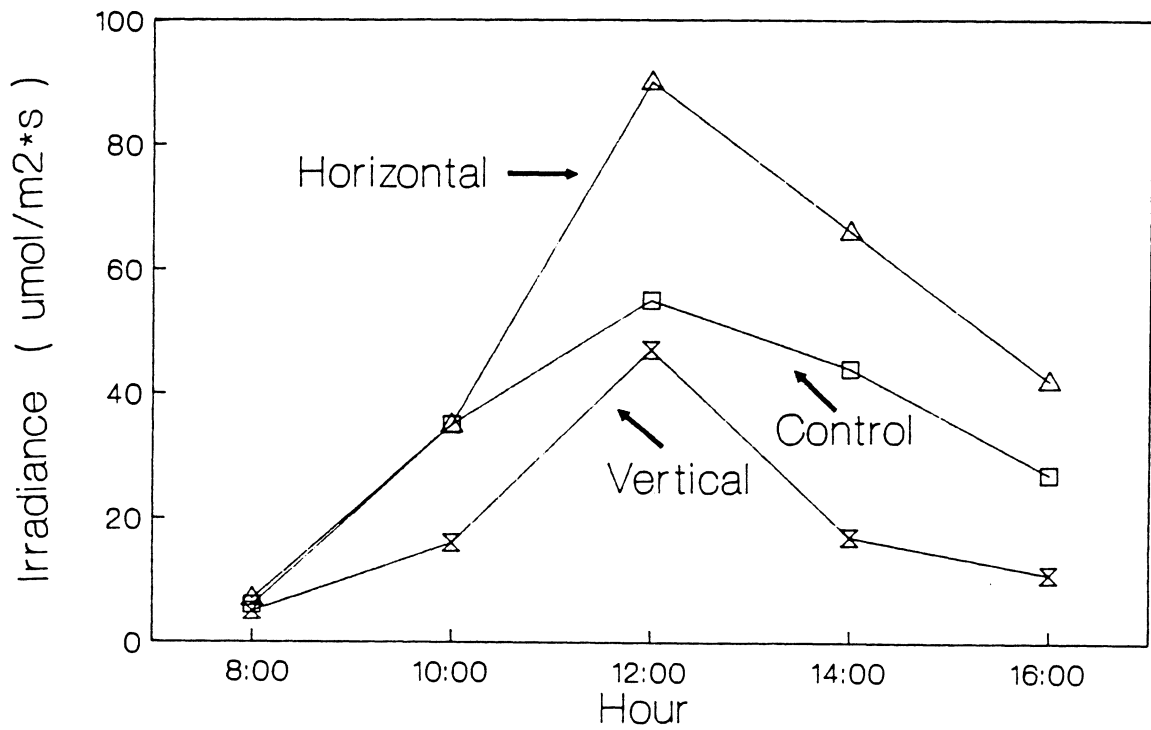
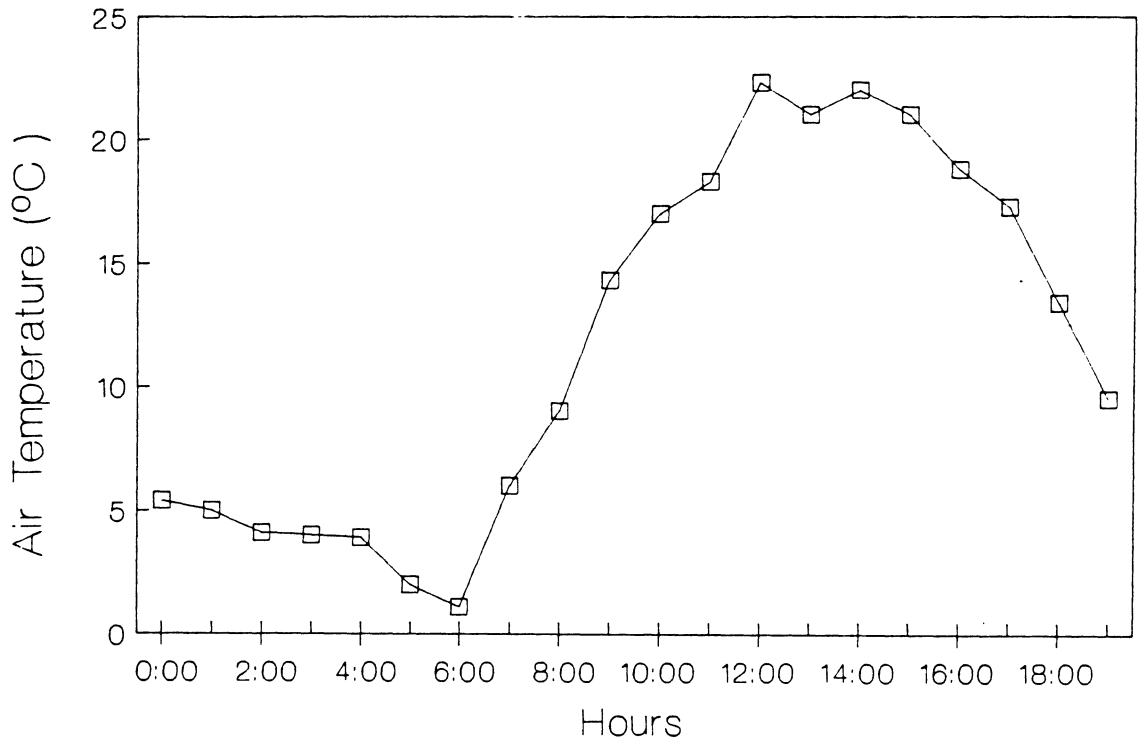


Figure 4. Diurnal course of irradiance on leaf surfaces: Symbols; (□), control leaves; (⊗), vertical leaves; and (Δ), horizontal leaves. Measurements were made on September 25, 1985.



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Figure 5. Diurnal course of air temperature on April 10, 1986.

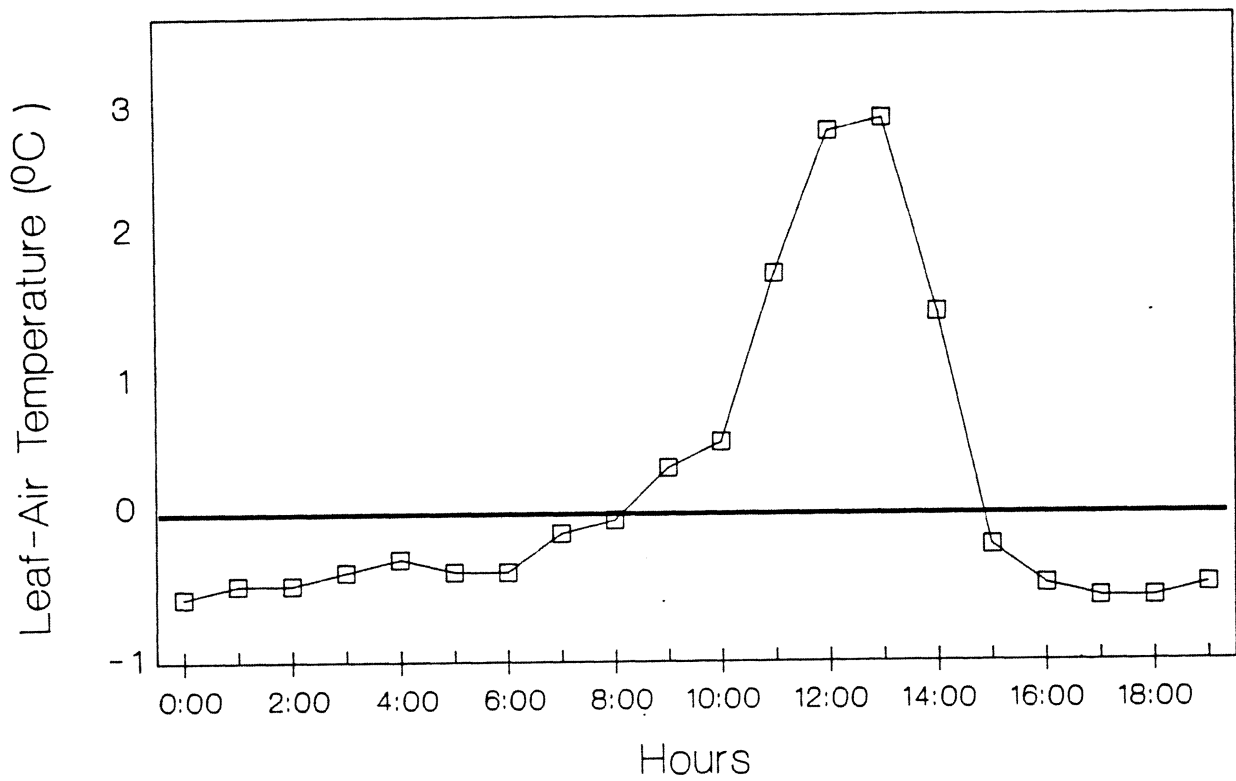


Figure 6. Diurnal course of leaf temp. minus air temp. on April 10, 86: Leaf temperatures were the average of six treatments ( control; curl free & vertical; curl free & horizontal; flat & angle move free; flat & vertical; and flat & horizontal).

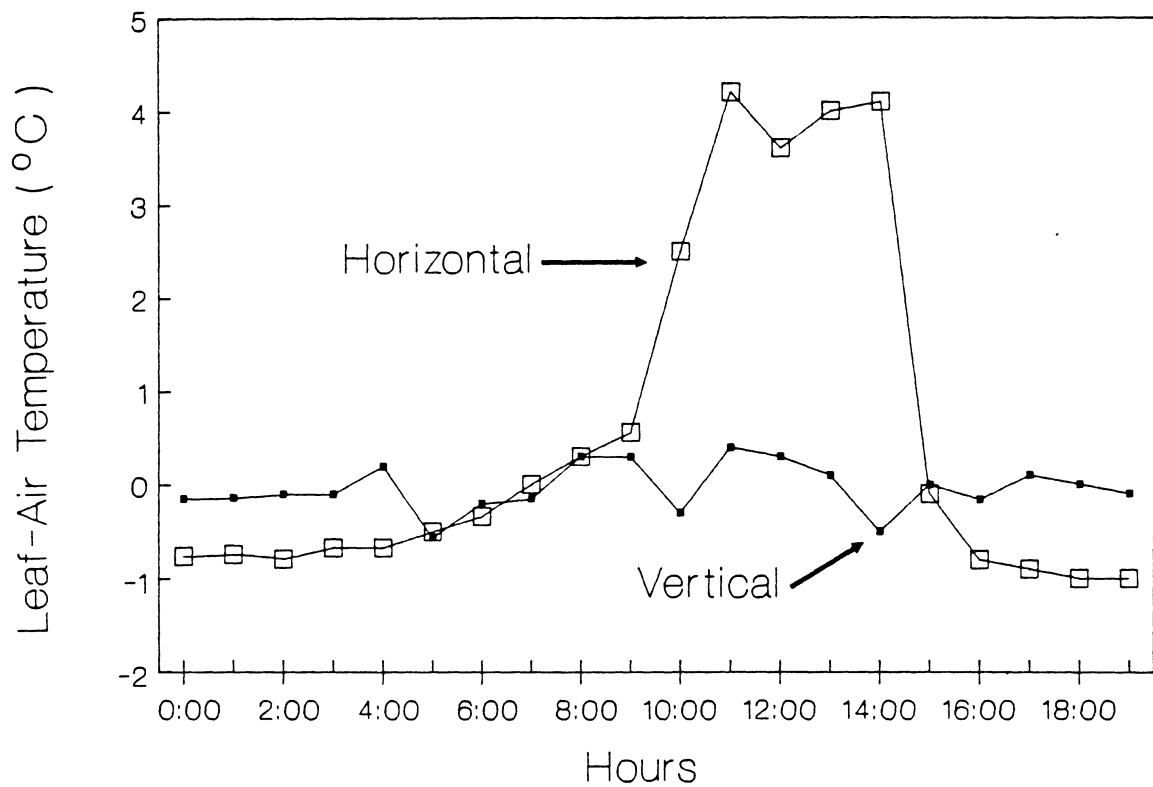


Figure 7. Diurnal courses of temperatures of vertical vs horizontal leaves: Measurements were made on April 10, 1986.

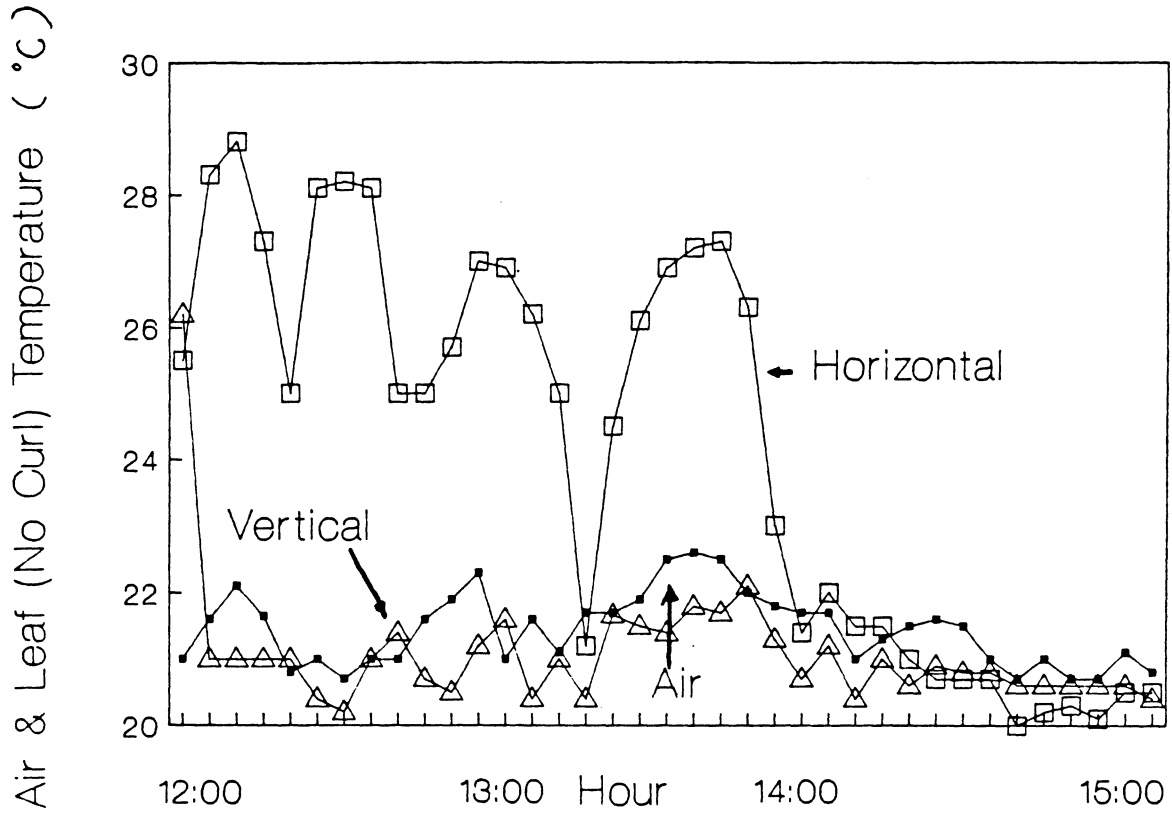


Figure 8. Short term ( 5 min. ) flat leaf and air temperatures: Symbols denote: (  $\Delta$  ): restrained flat & vertical; (  $\square$  ): restrained Flat & horizontal. Measurements were made on April 10, 1986.

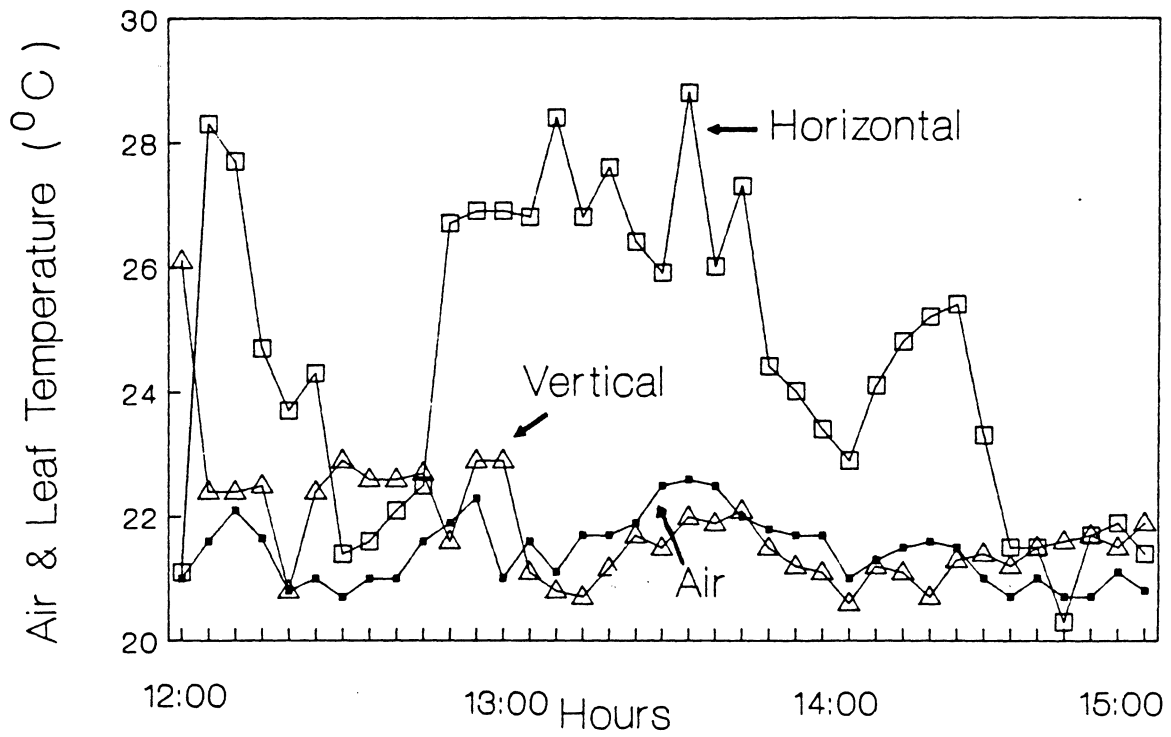
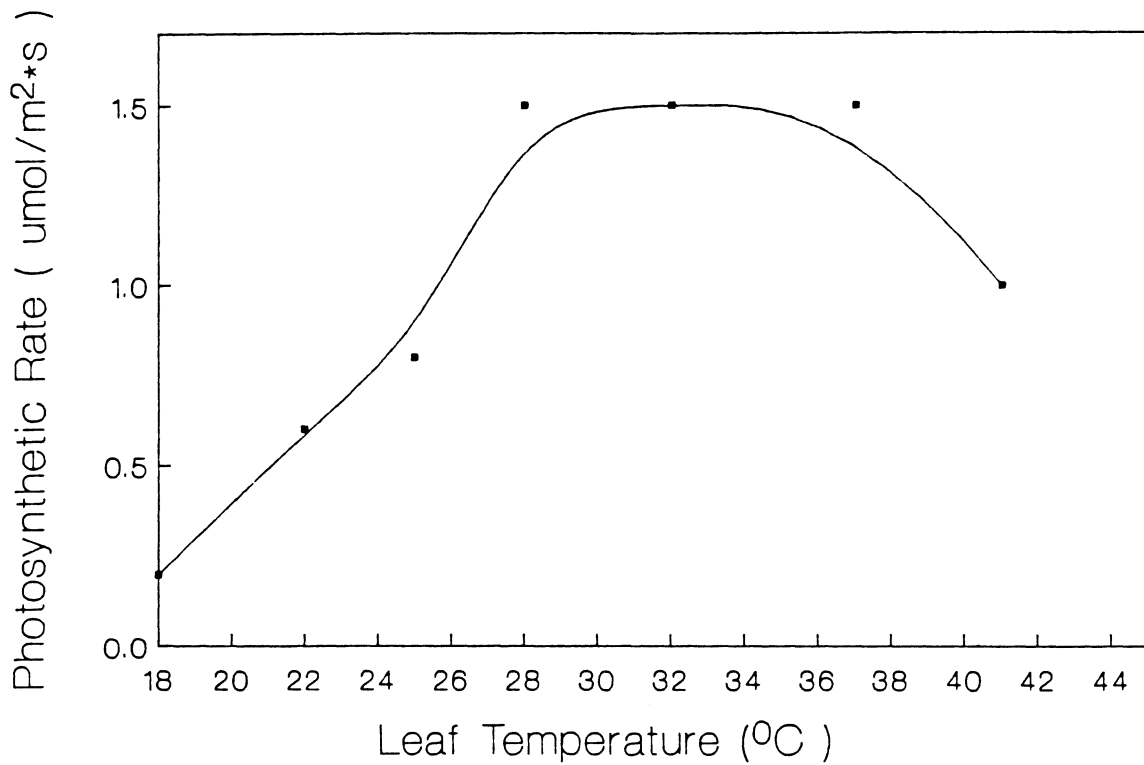


Figure 9. Short term ( 5 min. ) curl free leaf and air temperatures: Symbols denote: ( $\Delta$ ): curling free & restrained vertical; ( $\square$ ): curling free & restrained horizontal. Measurements were made on April 10, 1986.





**Figure 10.** Temperature response of net photosynthesis: Measurements were made insitu with intact leaves ( age: 2 yrs ), at  $700 \mu\text{mol}/\text{m}^2\cdot\text{s}$  ( PAR ),  $350 \mu\text{mol}/\text{mol}$   $\text{CO}_2$ , 21%  $\text{O}_2$  and a vapor pressure deficit of less than 10 mbar.

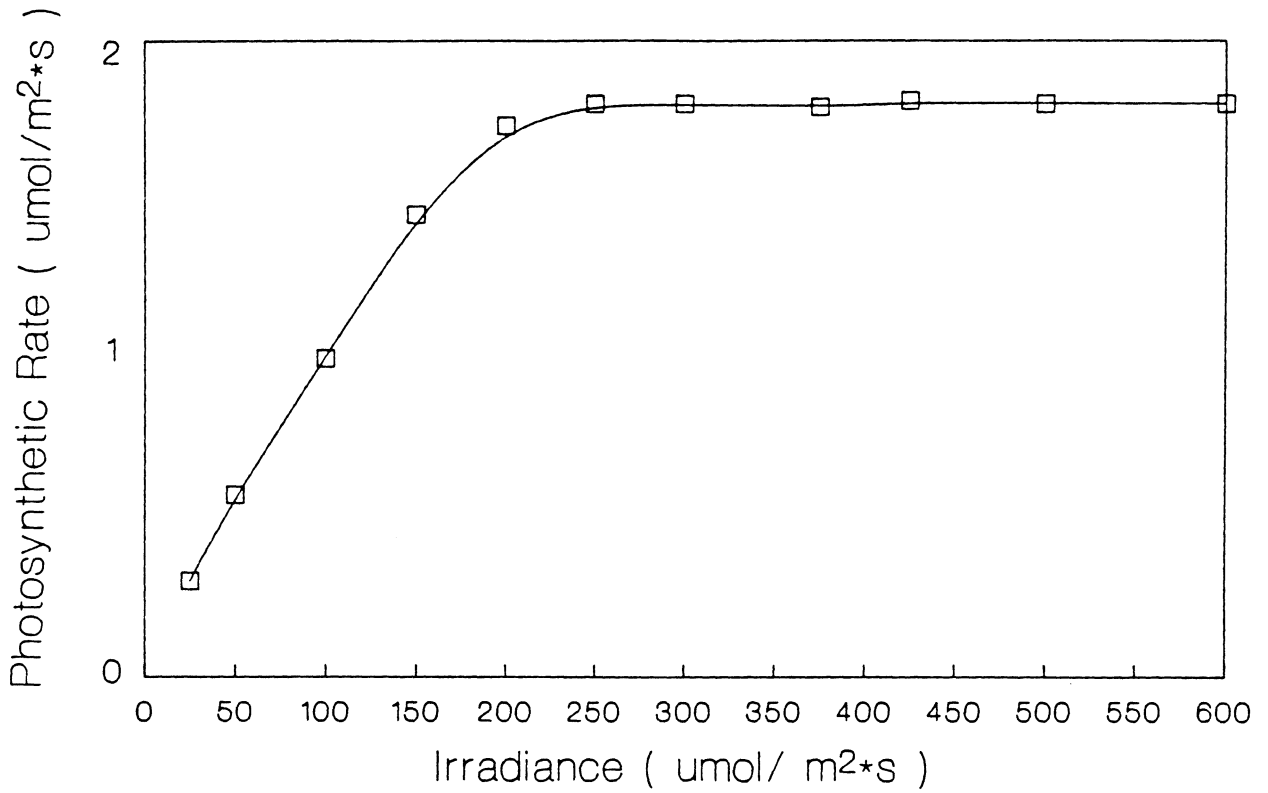


Figure 11. Light response of photosynthesis: Measurements were made insitu with intact leaves (age: 2 yrs ), at a leaf temperature of 30 c, 350  $\mu\text{mol}/\text{mol}$   $\text{CO}_2$ , 21 %  $\text{O}_2$  and a vapor pressure deficit of less than 10 mbar.

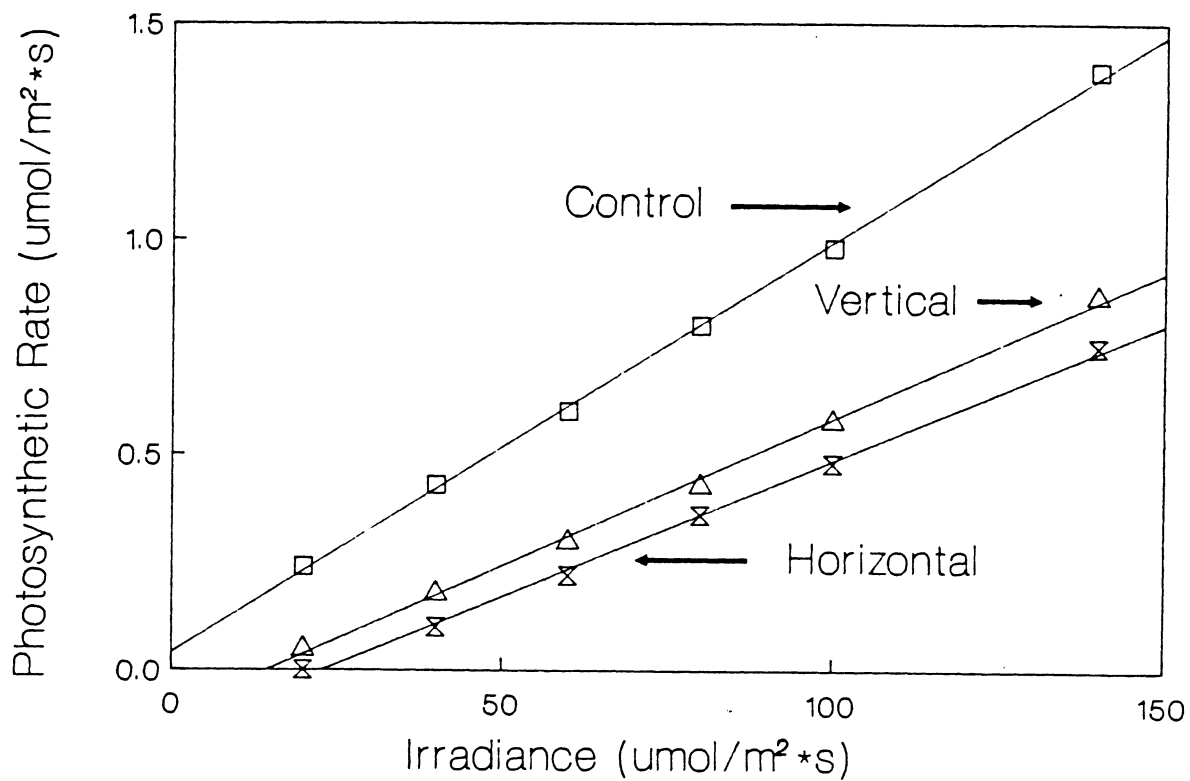


Figure 12. Linear-relationship between photosynthesis and irradiance ( 0 to 150 umol/m<sup>2</sup>\*s PAR )  
 Symbols denote; ( □ ): control leaves; ( △ ): vertical leaves; ( ⋈ ): horizontal leaves.

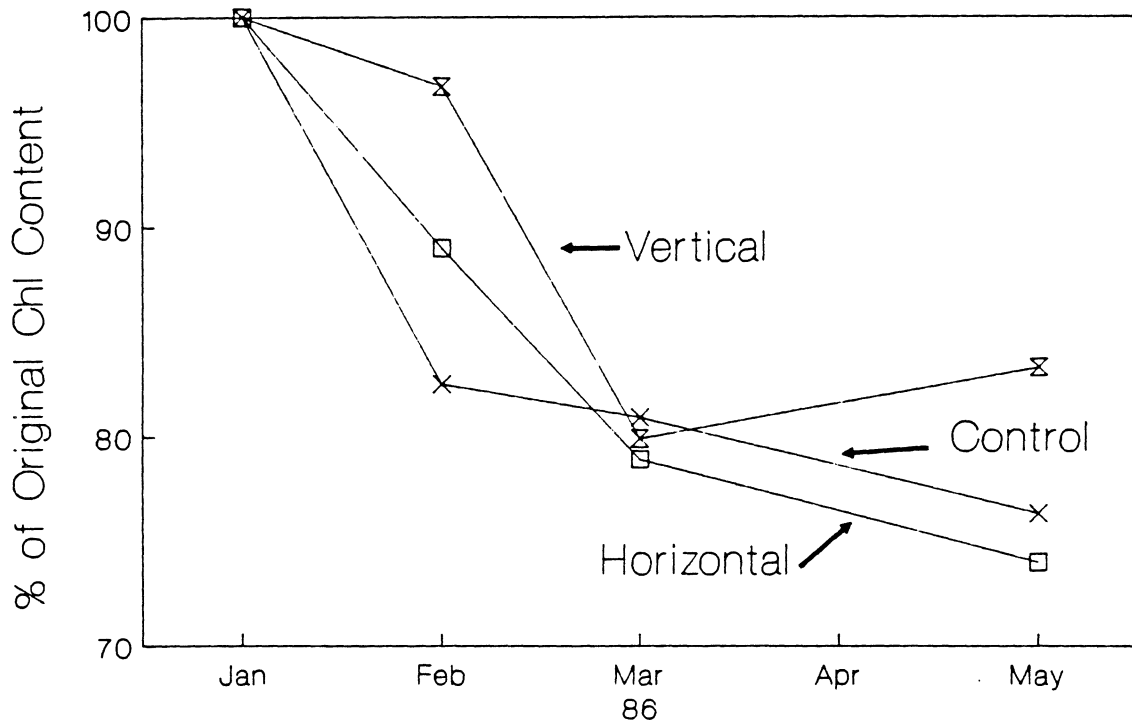


Figure 13. Comparison of relative chlorophyll contents ( % ): February 12, 86 values are taken as 100 %. Symbols denote: —x— control leaf —x— vertical leaf —x— horizontal leaf

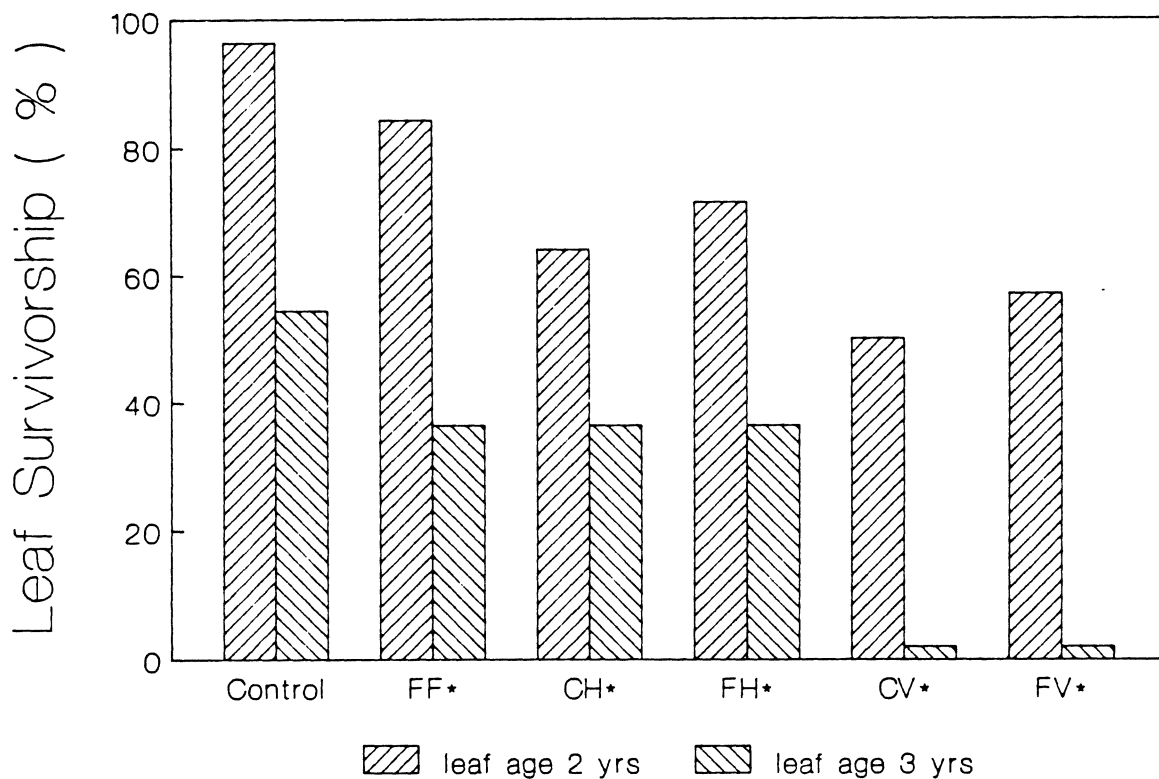


Figure 14. Comparison of relative leaf survivorship among treatments ( % ): Symbols denote: ( ▨ ): age 2 leaves; ( ▩ ): age 3 leaves; FF\*: flat & angle move free; CH\*: curling free & vertical; FH\*: flat & horizontal; CV\*: curling free & vertical; FV\*: flat & vertical leaves.

Table 1. Summary of photosynthesis, conductance, water use efficiency and intercellular CO<sub>2</sub> concentration

Treat	Cont	VC	HC	FF	VF	HF
A max* umol/m <sup>2</sup> s	1.81	1.20	1.03	1.17	1.08	1.33
se	.07	.40	.14	.12	.18	.12
conductance at Amax mmol/m <sup>2</sup> *s	53.4	32.2	32.2	32.8	43.4	59.3
se	7.4	11.6	5.9	5.9	8.6	4.1
WUF at Amax mol/mmol	1.4	1.3	1.5	1.9	1.0	1.0
se	.40	.10	.50	.30	.30	.10
Ci* at Amax umol/mol	283	288	287	262	304	311
se	17.0	3.0	16.0	20.0	9.0	7.0

**Table 2. Summary of the classes of total chlorophyll contents**

Treats	Con	CV	CH	FF	FV	FH
Time						
Jan.	A	A	A	A	A	A
Feb.	B	A	AB	B	AB	AB
March	A	AB	B	B	AB	AB
May	ABC	AB	BC	ABC	A	C

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