Morphological, Anatomical, and Physiological Changes of Orchardgrass Leaves Grown under Fluctuating Light Regimes

Pablo L. Peri,* Derrick J. Moot, Peter Jarvis, David L. McNeil, and Richard J. Lucas

ABSTRACT

The physiological and anatomical adaptability of pastures growing under trees in silvopastoral systems can alter the efficiency of conversion of energy to dry matter (DM). This study was conducted to determine the effects of different fluctuating light regimes (from 24 to 100% transmissivity) on leaf physiology, morphology, anatomy, and structure of orchardgrass (*Dactylis glomerata* L.) in a silvopastoral experiment (New Zealand). Slatted shade structures created a bimodal light regime that represented an existing silvopastoral system. Morphologically, as transmissivity decreased the length of the youngest fully expanded leaf and pseudo-stem height increased by up to 33% and the leaf width declined up to 22%. Physiologically, leaf adaptation to different light regimes was characterized by: (i) the light-saturated rate of net photosynthesis (Pmax) and to less extent the photosynthetic efficiency (α) in sun conditions was double; (ii) in sunny conditions plants grown under shade were photosynthetically less efficient than plants grown in full sunlight with lower Pmax and α values; (iii) when plants were exposed to severe shade, leaves adapted to severe shade condition had the highest Pmax, α, and θ, and saturated at the minimum photosynthetic photon flux density (PPFD) value. These changes in leaf photosynthesis characteristics under different light regimes were attributed to anatomical changes that caused reductions in stomatal conductance (gs), the mesophyll surface area/leaf surface area ratio (A_{mes}/A) and maintenance respiration for shade adapted plants. These photosynthetic responses and leaf adaptations to fluctuating light regimes can be included into a canopy photosynthesis model to improve the accuracy of DM predictions in silvopastoral systems.

ALTERNATING PERIODS of full sunlight and severe shade provide silvopastoral systems with a unique light regime compared with open pastures. In adapting to this, pasture plants may change the morphological and physiological characteristics of individual leaves. The research hypothesis is that the extent of shading in a fluctuating light regime can alter the efficiency of conversion of energy to dry matter (DM) by affecting light interception and the photosynthetic activity of individual leaves as a consequence of leaves adaptations. Together this will affect the productive potential and possibly the nutritive values of the pastures grown in silvopastoral systems (Varella et al., 2001). Thus, an understanding of changes induced by different light regimes at the leaf level will assist the development of predictive pasture production models, and in developing pasture management strategies for silvopastoral systems.

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In most cases, morphological adaptations of grasses to low light intensities result in longer and narrower leaves with higher specific leaf area to maximize light interception (Devkota et al., 2000). Furthermore, leaves photosynthetically acclimate to sun and shade conditions through both anatomical and physiological changes. Leaves grown in full sun have higher photosynthetic capacities per unit area, due to increased quantities of enzymes and higher stomatal conductance (gs) (Boardman, 1977) than shade leaves. Increases in photosynthetic capacity of leaves in response to a reduction in the light environment may result from an increase in the chlorophyll/protein ratio, lower chlorophyll *a*/*b* ratio, and higher quantum yields of shaded plants (Sims and Pearcy, 1989).

The effect of different continuous light intensities on leaf photosynthesis, related to anatomical and cell structural adaptations, has been reported for several species (Wilson and Cooper, 1969; Chabot and Chabot, 1977; Ward and Woolhouse, 1986; Nii and Kuroiwa, 1988; Sims and Pearcy, 1992; Niinemets and Tenhunen, 1997). Leaves adapted to full sunlight conditions are generally thicker due to an increase in mesophyll cells size (Charles-Edwards et al., 1974) and they have a greatest mesophyll surface area/leaf surface area ratio (Chabot and Chabot, 1977) compared with leaves grown under severe shade. These adaptations play an important role in the amount of light absorbed by a leaf and the diffusion pathway of CO_2 through its tissue that affects the photosynthetic rate of leaves (Syvertsen et al., 1995). Also, anatomical variables such as epidermal cell length may explain the elongation of leaf length (Forde, 1966) and the decrease mean leaf angle of pastures grown under shade to increase their capacity of light interception.

However, the physiological and anatomical adaptability of leaves to a fluctuating light environment, related to the net photosynthesis of pastures growing under trees in silvopastoral systems, has received little attention. Also, the relationships between shade and other environmental factors (temperature and water stress) affecting the adaptability of orchardgrass leaves in a silvopastoral system, have not been defined.

Of interest in the present study are the relationships between environmental controls on morphological, anatomical and physiological adaptations in response to fluctuations in light, which differ from those operating

Abbreviations: ANOVA, analysis of variance; DBH, diameter at breast height; DM, dry matter; ESE, standard error of the estimate; gs, stomatal conductance; H, pseudo-stem height; IRGA, infrared gas analysis system; L, length; LAI, leaf area index; LSD, least significant difference; P_{max}, net photosynthesis; PPFD, photosynthetic photon flux density; R/FR, red to far-red wavelengths; RUE, radiation use efficiency; SEM, standard error of means; SLA, specific leaf area; *T*, temperature; W, width; ψ_{lp}, predawn leaf water potential.

under steady-state conditions (Pearcy et al., 1996; Peri et al., 2002b). The objectives of this study were to (i) evaluate the effect of different light intensities from fluctuating light regimes on leaf morphology and anatomy in conjunction with seasonal changes in other environmental factors (water stress and temperature); (ii) determine the effect of fluctuating light regimes on leaf photosynthesis and leaf structural adaptations when temperature, water, and N content were nonlimiting; and (iii) relate the photosynthesis response with the morphological, anatomical, and physiological adaptations to fluctuating light regimes.

MATERIALS AND METHODS

Experimental Site

The silvopastoral experiment was located at Lincoln University, Canterbury, New Zealand (43°38′ S and 172°28′ E) on a Templeton silt loam (UdicUstochrept, USDA Soil Taxonomy) soil that consists of 1 to 2 m of fine alluvial sediments over gravels. The climate is described as temperate and subhumid with a long-term rainfall average of 660 mm and annual potential evaporation of 1300 mm. Mean annual temperature is 11.4°C. No fertilizer or lime were applied to the experimental area since its establishment. Soil tests in September 1999 indicated low soil fertility (pH 5.9, Olsen P 7.5 μg mL^{-1}, $S(SO_4)$ 3.5 ppm) for this region.

Monterey pine (*Pinus radiata* D. Don) trees were planted in July 1990 at 1000 stems ha^{-1} (1.4 m within rows and 7 m between rows) covering a total of 5.2 ha. Trees were periodically thinned to the final tree stocking of 200 stems ha^{-1} (7 m within rows and 7 m between rows). At age 10, trees had a mean total height of 13.3 m, a diameter at breast height (DBH) outside bark of 0.26 m, and a mean crown length (derived by subtracting pruned from total height) of 7.3 m.

Orchardgrass was sown in September 1990 in three 46.2 by 42.0 m (0.194 ha) main experimental units. At the same time three orchardgrass main experimental units (27.5 by 18 m) were established in an adjacent open site. These areas have been grazed by sheep since 1993. The usual regime has been for the three orchardgrass plots to be rotationally grazed with a rotation length of 28 ± 2 d (grazed for 7 d after 21-d regrowth period), but with no grazing in winter (May–August). These experimental units are part of a larger silvopastoral experiment described by Mead et al. (1993). During the spring summer grazing period the mean area covered by visually obvious urine patches varied from 25 to 32%. Patches had a mean diameter of 0.22 m and with from 173 to 448 kg N ha^{-1} depending on the season (Peri et al., 2002c). No samples from these urine patches were taken for measurements.

Experimental Design

Within each of the three main orchardgrass experimental units of the silvopastoral experiment, a study plot of 14.0 by 5.0 m was located in the middle of the 7.0-m wide inter-row under trees. These were also created in the adjacent open pasture experimental units. Within each study area, a slatted shade structure measuring 3.0 by 2.1 m covered with pine wood slats (150 mm wide) and gaps between slats (150 mm wide) was used to reduce the total incidence of light by approximately 50%. This structure provided a bimodal light regime that represented the existing silvopastoral system (Peri et al., 2002b). The shade structures were supported horizontally on a vertically adjustable metal frame, which allowed the shade source to be maintained at 0.3 m above the orchardgrass canopy. For the slatted shade structure, the objective was to create intervals of sunlight and shade similar to the shade pattern of the radiata pine in the silvopastoral area.

This experiment was arranged with open (100% transmittance) and silvopastoral (~58% transmissivity) plots as main treatments with three replicates. Within each replicate a orchardgrass plot was split into two further subplots: slatted shade and no slatted shade. This gave four light transmission regimes: (i) orchardgrass open pasture (O), (ii) orchardgrass open pasture under slatted shade (O+S), (iii) orchardgrass pasture under tree shade (T), and (iv) orchardgrass pasture under trees and a slatted shade (T+S). The T+S treatment was included as representative of the extended periods of shade that a pine tree silvopastoral system with more developed tree crowns would experience.

The slatted shade structures were orientated in an east–west direction in the main plots with the slats north–south. They were set up continuously in the plots from September 1999 to May 2001. During periods when main plots were grazed, the shade frames were removed to avoid damage on plants through sheep using the structures as a camping area. Immediately after each grazing, plots were trimmed with a mower to an even height of 20 mm and slatted frames were replaced in their original positions.

Environmental Measurements

Air temperature measurements were taken onsite in the open and under trees using a digital temperature sensor (TDC-01A, Monitor Sensors, Queensland, Australia) located 1.5 m aboveground, which logged every 6 min (resolution ±0.2°C). The mean daily temperature was similar in the open and under trees. In both summers (December–February 1999–2000 and 2000–2001), the mean temperature under trees was 0.4°C warmer than in the open, and during winter (June–August 2000) it was 0.2°C warmer. However, during a sunny day in autumn–winter (maximum temperatures between 10 and 15°C) the temperature under trees was up to 3°C warmer at midday and morning (from 500 h), but the difference was reversed after sunset. In contrast, during sunny hot days in summer (>28°C) there was minimal difference in air temperature under trees and open pasture sites.

Light intensity was monitored with quantum sensors (Li-Cor LI-191SB, Lincoln, NE) installed above and below the slatted shade structures, but above orchardgrass canopy height. These measured the photosynthetic photon flux density (PPFD) in the 400- to 700-nm waveband every 5 min and a datalogger recorded mean PPFD at 30-min intervals. The daily PPFD was integrated to calculate the accumulated monthly photosynthetic photons per unit area. The mean maximum photosynthetic photons reaching the open orchardgrass pasture was in December (1715–1815 mol m^{-2}) corresponding to the maximum 1200 h solar angle elevation (69.8° at 1200 h). The minimum (302 mol m^{-2} for open pastures) was in midwinter (June) with the lowest 1200 h solar angle elevation of 23°. In December, pastures in the open received 720, 960, and 1220 mol photons m^{-2} more than pastures under trees, O+S and T+S, respectively. The daily PPFD integral in the open for a sunny day in spring or autumn (e.g., 21 September or 21 March at solar angle elevation of 46.5° at 1200 h), summer (21 December at solar angle elevation of 69.8° at 1200 h) and winter (21 June at solar angle elevation of 23.0° at 1200 h) were used as a reference (100% transmissivity) to calculate the transmissivity of the shade treatments (Table 1). This was used to represent the relative reduction of photosynthetic photons in the shaded treatments compared with the open pasture. The total daily integral photosynthetic photons received in open pasture

Table 1. Relative transmissivity (%) of the shaded treatments as a percentage of the open daily integral photosynthetic photon flux density (PPFD) for sunny days at three different solar angles elevation (seasons) in Canterbury, New Zealand. Values in parentheses are the total daily integral of PPFD for open expressed as mol photons m^{-2} d^{-1}.

Solar angle at 1200 h	69.8°	46.5°	23.0°
Treatments	**Summer**	**Autumn–Spring**	**Winter**
Open	100% (63.3)	100% (36.0)	100% (10.6)
Open + slats	45%	43%	41%
Trees	62%	60%	55%
Trees + slats	26%	25%	23%

around the 21 December (63.3 mol photons m^{-2} d^{-1}) were six times higher than in winter (21 June) (Table 1). The transmissivity of the shaded treatments decreased with a decrease in solar angle elevation from summer to winter. The light regime measured from treatments throughout a summer sunny day is shown in Fig. 1. The length of alternating periods of full sunlight (1800–1900 μmol m^{-2} s^{-1} PPFD at midday) to severe shade (120–130 μmol m^{-2} s^{-1} PPFD) under the 10-yr-old trees (Fig. 1a) was 45 to 60 min from 08.00 to 11.00 h and 17.00 to 20.00 h, but this increased to 90 to 120 min around midday. The interval of full sunlight and shade periods around midday for the T+S treatment was approximately 75 and 180 min, respectively (Fig. 1c).

Spectral irradiance from 300 to 1100 nm wavelengths was measured with a Li-Cor LI-1800 spectro-radiometer (Lincoln, NE). Measurements were taken at 1200 h and 1700 h for a sunny day in spring, which corresponded to solar angle elevations of 46.5° and 17.6°, respectively. From the total spectral irradiance data, proportions of red (660 nm) to far-red (730 nm) wavelengths (R/FR) were calculated. The R/FR ratio decreased from sun to any of the shaded situations (Table 2). The minimum value of R/FR was 0.54 at 1200 h in the middle of the tree shade. The R/FR also decreased under the shade of slats. There was no difference in R/FR for two different solar angles elevation (1200 h and afternoon) for full sunlight conditions. However, under the tree shade, the R/FR increased at the lowest solar angle.

Water Status and Morphological Measurements

Predawn leaf water potential (ψ_{lp}) was obtained from a random sample of five of the youngest fully expanded leaves from each treatment with a pressure chamber (Model 1002, PMS Instrument Co., Corvallis, OR) on monthly measurements. These measurements were summarized on as a seasonal basis: winter (June–August), spring (September–November), summer (December–February), and autumn (March–May).

Morphological measurements were taken on a random selection from 20 dominant tillers per plot on each measurement date after 21 d regrowth. Dominant tillers were defined as those positioned at the top of the canopy. From these the length and area of the youngest fully expanded leaf (with visible ligule), pseudo-stem height (height of the sheath from the above-ground soil level up to the ligule of the youngest expanded leaf), lamina width at midposition, and number of green leaves per tiller were measured.

The seasonal effect of light regimes, water status, and temperature on monthly morphological measurements was analyzed by multiple regression. This enabled the relative impact of each environmental factor on morphology to be separated seasonally. The coefficient of determination (r^2) and standard error of the estimate (ESE) of the morphological variables were used to quantify the "goodness of fit" for linear equations.

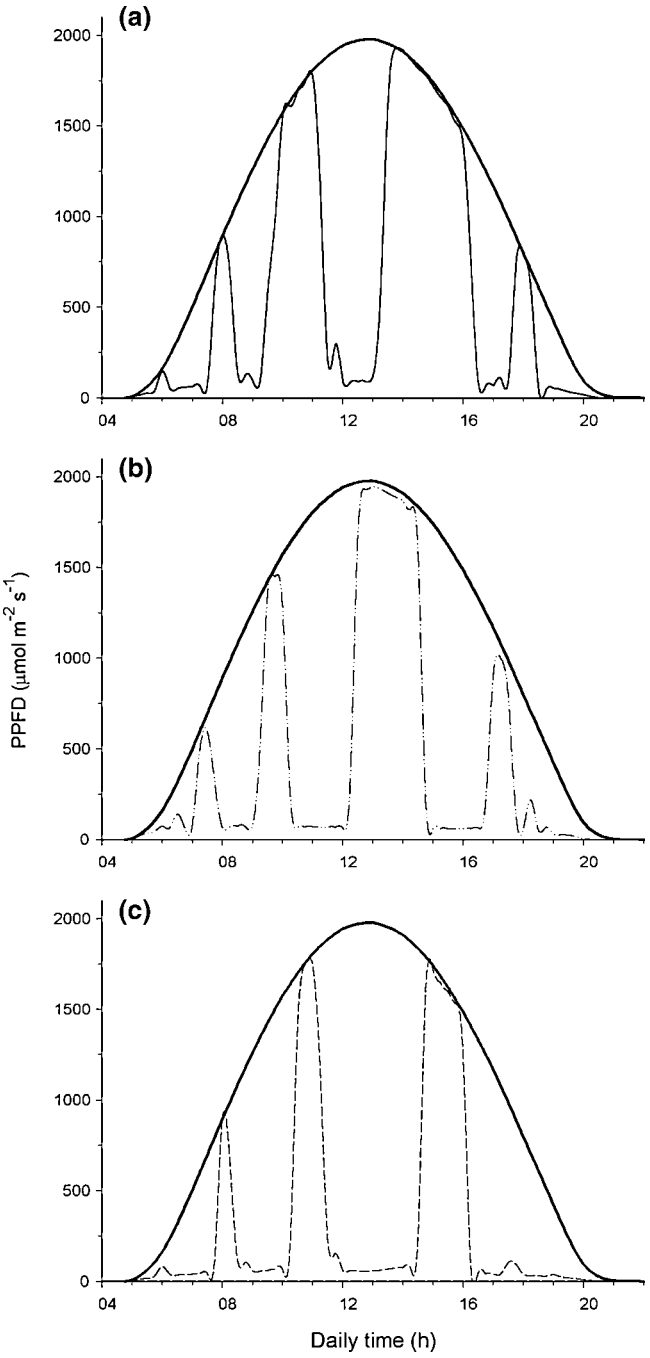

Fig. 1. Photosynthetic photon flux density (PPFD) received on a typical sunny summer day (23 Dec. 1999) in Canterbury, New Zealand for orchardgrass in the open (thick line) and (a) under trees (thin line), (b) under a slat structure in open (dotted and dashed line), and (c) under trees plus the slat structure (dashed line).

Anatomical Measurements

Anatomical variables of the youngest expanded leaf were measured from the mid-point of the lamina (between the ligule and the leaf tip), which was considered to be representative of the blade as a whole. Measurements were taken on six occasions during 2 yr—spring when maximum growth occurred (November 1999 and 2000), summer (February 1999 and 2000) to evaluate the effect of water stress, and autumn (April 2000 and May 2001) to evaluate the recovery from water stress and

Table 2. Red (660 nm) to far-red (730 nm) (R/FR) ratio (dimensionless) at 1200 and 1700 h for a sunny summer day and for different light conditions.

Light condition	R/FR ratio at 1200 h (69.8° solar angle)	R/FR ratio at afternoon (1700 h) (17.6° solar angle)
Open sun	1.32	1.34
Open sun under slat	1.28	1.28
Open shade under slat	0.74	0.86
Tree sun	1.24	1.29
Tree shade (middle)	0.54	0.83
Tree shade under slat	0.40	0.58

impact of low temperatures. For each treatment 12 leaves were harvested, sealed in plastic bags, and immediately stored in a cold box. On the same day, in the laboratory, six leaves for each treatment were used for cross-section analysis and the other six leaves for longitudinal abaxial surface analysis. Tissue samples and protocol for leaf anatomical study was performed using sterological procedures described by Chabot and Chabot (1977) and Ward and Woolhouse (1986). Cross-section and longitudinal abaxial surface analyses were examined using a compound microscope (Olympus BH2) with 20× and 40× objective calibrated with an ocular micrometer. Images were captured using a video camera (JVC, Victor Company, Japan) mounted on top of the microscope with an adaptator (JVC AC-C6222).

Cross-Section

Cross-sections of 30 μm were made with a sliding freezing microtome (Leitz 1310, Ernst Leitz-Wetzlar, Germany). Leaf and epidermis thickness, and number and size of mesophyll cells were determined in the half-leaf width (midpoint of midrib to leaf margin) of the cross-sectional area. Height and width of 20 palisade cells and mean diameter of 40 spongy cells were also measured for each cross-section. The number per length cross-section and area of bundles, considering both vascular tissue and sclerenchyma, was measured using a square grid of test lines. This anatomical parameter was measured to relate with potential changes in the mean leaf angle of orchardgrass pasture grown under different light regimes.

Longitudinal Abaxial Surface

Anatomical measurements in the longitudinal abaxial surface of the youngest expanded leaf were performed around the midpoint area of the lamina (between the ligule and the leaf tip). Length and width of 60 epidermal cells were measured for each sample. These were measured because the expansion of the epidermis during leaf development may control the expansion of mesophyll and vascular tissue, and thus determine the final leaf growth in grasses grown in shaded conditions (Forde, 1966). This may provide an indication for the leaf length changes of orchardgrass plants grown under different light regimes. Epidermal cell width measurements were made across the widest point of cells in the same area as those measured for length. To estimate the number of epidermal cells per leaf abaxial area, epidermal cell area was assessed as length multiplied by 75% of the width due to its pronounced taper toward each end of orchardgrass cells (Forde, 1966).

Photosynthesis Measurements

The photosynthesis rate and stomatal conductance (gs) were measured on a random sample of the youngest fully expanded intact leaves on six different tillers from each treatment. All measurements were taken when water ($\psi_{lp} = -0.01$ MPa to -0.12 MPa, or -0.1 to -1.2 bar), N content (>40 g N kg^{-1} DM), and temperature (19–23°C) were not limiting (Peri et al., 2002a) during several days in October, November, and December 1999–2000. All measurements were taken at midday ± 1 h on sunny days, 21 d after grazing. For the three fluctuating light regimes (T, O+S, and T+S) measurements were taken after 120 min of severe shade when leaf photosynthesis reached a steady-state and after 45 min in full sunlight when the induction process (recovery of leaf photosynthesis from low to high irradiance) was completed (Peri et al., 2002b). Net photosynthesis (μmol CO_2 m^{-2} s^{-1}) and gs to water vapor (mol H_2O m^{-2} s^{-1}) were measured simultaneously in an open infrared gas analysis system (IRGAs) with the "LiCor 6400 Portable Photosynthesis System" instrument (Lincoln, NE). This system provides steady light, CO_2, H_2O, and temperature conditions for measurement. Net photosynthesis and transpiration are computed by measuring the airflow rate, the incoming and leaf chamber CO_2 and H_2O concentrations, and leaf area. Inside the leaf chamber, the programmed concentration of CO_2 was 400 μmol mol^{-1} and the leaf temperature was 21 ± 1°C (controlled by Peltier thermoelectric coolers). Light curves with seven light intensities (0, 100, 250, 500, 750, 1000, and 2000 μmol m^{-2} s^{-1} PPFD), were measured using the "Auto Light Curve Program." The minimum wait time used was 60 s for each light intensity, with a 3% coefficient of variation for each of these intensities.

The rate of net photosynthesis as a function of PPFD is accurately described by a nonrectangular hyperbola (Marshall and Biscoe, 1980; Thornley, 1998). Therefore, the net photosynthesis rate measurements from light curves were used to fit nonrectangular hyperbola functions (Eq. [1]) that have the mathematical form:

$$P_n = \frac{1}{2\theta}\left\{\alpha I_1 + P_{max} - \left[(\alpha I_l + P_{max})^2 - 4\theta\alpha I_l P_{max}\right]^{\frac{1}{2}}\right\} \quad [1]$$

where P_n is the rate of single leaf net photosynthesis (μmol CO_2 m^{-2} s^{-1}), I_l is the irradiance incident on a leaf (μmol m^{-2} s^{-1} PPFD), α is the initial slope of the light-response curve or photosynthetic efficiency, also referred to as the quantum yield or photochemical efficiency (μmol CO_2 μmol^{-1} PPFD), θ is the degree of curvature or convexity (dimensionless), and P_{max} is the light-saturated rate of net photosynthesis or the asymptote of the curve (μmol CO_2 m^{-2} s^{-1}). Estimation of dark respiration was obtained from light curves at zero PPFD. The saturation point was also estimated from these curves. Overall, 80 photosynthesis light-response curves (20 for each light regime) were fitted to determine the effect of leaf adaptation to fluctuating light regimes on P_{max}, α, and θ.

Adaptations Related to Leaf Photosynthesis

Simultaneously with photosynthesis measurements, leaf N content, leaf chlorophyll, stomatal frequency, and the length of individual stomata, individual leaf specific leaf area (SLA), the mesophyll surface area/leaf surface area ratio (A_{mes}/A), and mean leaf angle were measured or calculated to examine the potential causes of changes in leaf photosynthesis against leaf adaptations.

The N content of green leaves from a 0.2 m^2 quadrat in nonurine areas cut to 25-mm height was determined using the Kjeldahl technique. Samples were dried in a forced draft oven at 65°C for 48 h and ground in a mill containing a 1-mm stainless steel screen.

Leaf chlorophyll content was measured on a random sample of the youngest fully expanded intact leaves at mid position.

Chlorophyll was extracted from 2 cm^2 of fresh leaf on 60 leaves in 90% acetone after grinding the leaves in a mortar. Absorption was measured at 665 nm (chlorophyll *a*) and 645 nm (chlorophyll *b*) using a spectrophotometer (Unicam UV-Visible Spectrometry, Cambridge, UK). The total chlorophyll concentration (g m^{-2}) was calculated from the absorbance measurements using equations from Andrews et al. (1984).

Stomatal frequency per mm^2 (f) of abaxial leaf area and the mean length of 50 individual stomata (L) were measured from anatomical analysis. Total pore space was estimated by multiplying the mean stomatal length by the stomatal frequency ($f \times L$). This calculation was performed to examine the potential reasons for changes in gs for orchardgrass adapted to different light regimes.

The specific leaf area of the youngest fully expanded leaf was calculated from the measured leaf area and dry weight. Samples of six leaves per treatment were spread randomly over a transparent sheet and then scanned using a flat-bed scanner. The leaf area was determined using imageanalysis software (DT-Scan, Delta-T Ltd., Cambridge, UK). Leaves were dried in an oven at 65° for 24 h.

From the anatomical analysis, the mesophyll surface area/leaf surface area ratio (A_{mes}/A) was calculated by multiplying the mean surface area of palisade and spongy cells by their number in units of leaf area (10^4 μm^2). The surface area of palisade cells was calculated as the area of a cylinder considering the height and width of cells. The surface area of spongy mesophyll cells was calculated as the area of a sphere using the mean diameter and considering closely packed cells.

The Li-Cor LAI-2000 Plant Canopy Analyser (Lincoln, NE) was used to measure the mean canopy leaf angle (mean tilt angle, MTA) as described by Welles and Norman (1991) and leaf area index (LAI). There are difficulties in measuring total LAI for grasses because the optical sensor head of the instrument is 40 mm high. Therefore, aluminium trenches 40 mm deep by 30 mm wide by 1.2 m long were set up for all treatments so that the top of the sensor was at the soil surface. In this study, measurements were taken from one reading above the canopy followed by five readings beneath, along the trench (transect). As the Li-Cor LAI-2000 requires diffuse light to give reliable measurements, the instrument was only used under uniform overcast conditions, or before sunrise and after sunset. To avoid contamination of the measurements by the operator, a 180° view cap was used.

Analyses

Statistical analyses were performed using the Genstat statistical package (Genstat 5, 1997). Standard error of means (SEM) were used to calculate least significant differences (LSD) at the 0.05 probability level for means separation of the variables. Significant differences for the experiment with four light regimes were determined for each rotation by analysis of variance (ANOVA) according to the split-plot design with three replicates. Variables were also analyzed by considering time as a factor. Thus, this analysis was performed to detect potential interactions between a pasture variable (such as tiller morphology) and the main environmental factors (such as temperature), which vary with time (seasons).

RESULTS

Water Status

In spring and winter, ψ_{lp} was always above −0.15 MPa (−1.5 bar), indicating that the treatments were not moisture stressed during those periods (Fig. 2). However, in summer and autumn of both years, pastures were under water stress. On average, ψ_{lp} for pastures under trees was −0.18 MPa (−1.8 bar) more negative than for open pastures. The shaded treatment O+S had a higher ψ_{lp} than the adjacent open pasture. Similarly, the treatment T+S had a higher ψ_{lp} than the pasture under trees.

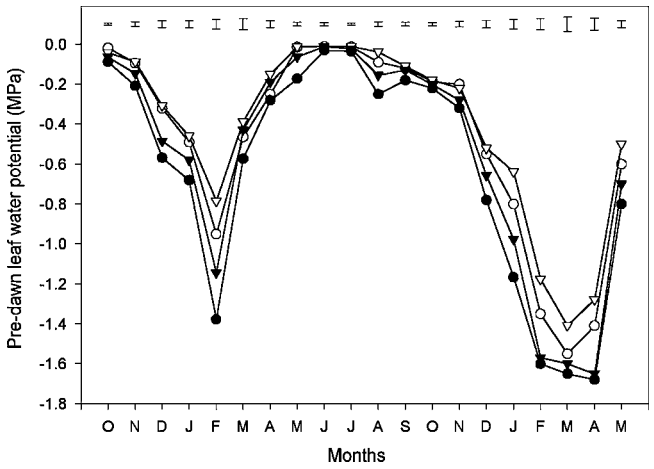

Fig. 2. Mean predawn leaf water potential for four shaded treatments: Open (open circle) (100% transmissivity), open + slats (open triangle) (~43% transmissivity), under trees (closed circle) (~58% transmissivity), and trees + slats (closed triangle) (~24% transmissivity) over 2 yr. Bars indicate standard error of the mean (SEM).

Tiller Morphology

The light regime affected the tiller morphology of orchardgrass plants. The length of the youngest fully expanded leaf (Fig. 3a) and pseudo-stem height (Fig. 3c) increased ($p < 0.05$) with decreasing light intensity. In contrast, the youngest fully expanded leaf was wider ($p < 0.05$) for orchardgrass plants grown in full sunlight conditions (Fig. 3b). There was an interaction ($p < 0.05$) between treatments and time as expressed by seasonal fluctuations in tiller morphology. The greatest ($p < 0.01$) leaf and pseudo-stem elongation occurred during October and November when moisture was nonlimiting (Fig. 2). In contrast, there was less leaf extension in summer (January–February) when water was limiting and in winter (June–July) when temperature dropped. In autumn significant rainfall reduced the ψ_{lp} in March 2000 and May 2001 (Fig. 2), and there was a subsequent recovery in leaf expansion for all treatments. The light regime had no effect ($p = 0.12$) on green leaf number in any season with a mean of 2.4 ± 0.28 in all treatments.

There was a strong relationship between mean morphological measurements (length [L] and width [W] of the youngest fully expanded leaf and pseudo-stem height [H]) and both mean monthly daily temperature (T) (T: range from 6 to 18°C), mean monthly ψ_{lp} (range from −0.01 to −1.7 MPa, or −0.1 to −17.0 bar) and monthly PPFD for the different light regimes (range from 69 to

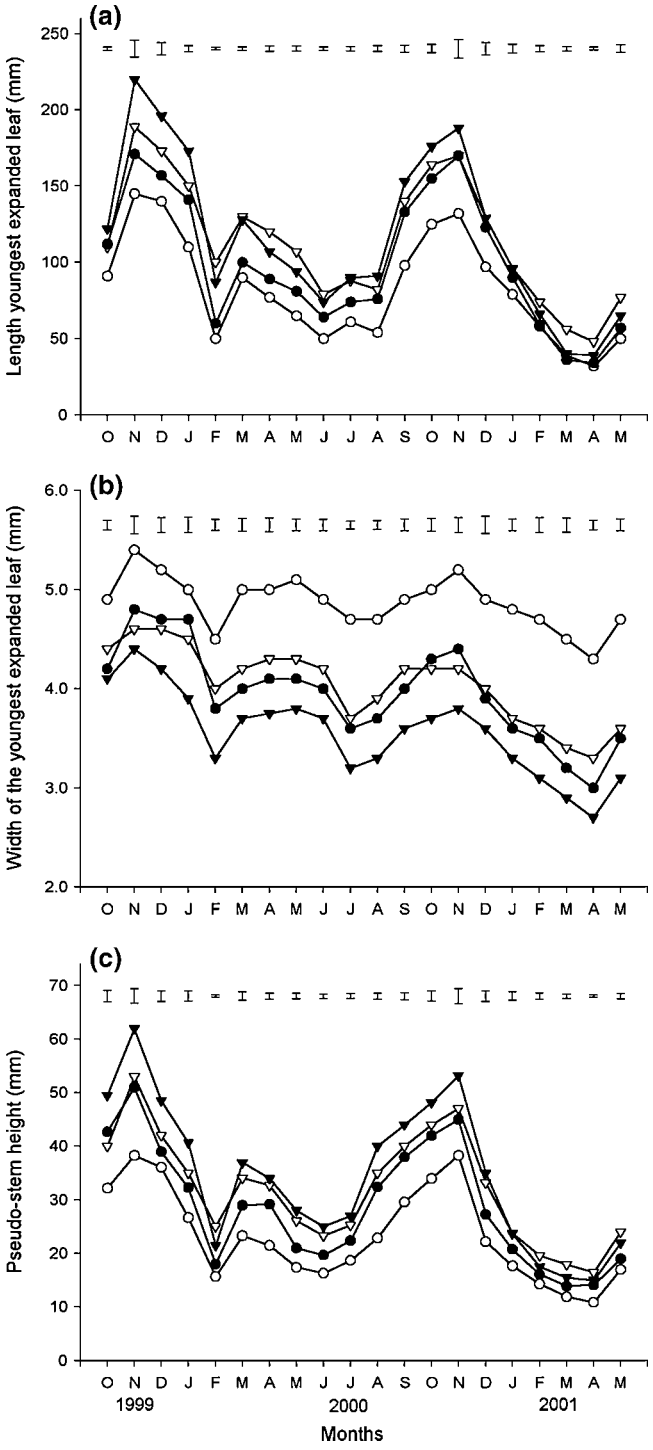

Fig. 3. (a) Mean length of the youngest fully expanded leaf, (b) mean lamina width at midposition, and (c) mean pseudo-stem height (height of the sheath from the aboveground soil level up to the ligule of the youngest expanded leaf) of dominant orchardgrass tillers grown at 100% (open circle), ~43% (open triangle), ~58% (closed circle), and ~24% (closed triangle) transmissivity. Bars indicate standard error of the mean (SEM).

1820 mol m^{-2} mo^{-1}). These were described empirically by fitting multiple linear functions:

$$L = 17.29 - 15.2 \times 10^{-3}\ PPFD + 84.9\psi_{lp} + 11.35T\ (r^2 = 0.70;\ ESE = 30.5) \quad [2]$$

$$W = 3.87 + 0.00106\ PPFD + 0.657\psi_{lp} - 0.0061\ T(r^2 = 0.75;\ ESE = 0.25) \quad [3]$$

$$H = 16.69 - 4.1 \times 10^{-3}\ PPFD + 21.3\ \psi_{lp} + 11.35\ T(r^2 = 0.68;\ ESE = 6.11) \quad [4]$$

where L is the mean monthly length of the youngest fully expanded leaf (mm); W is the mean monthly width of the youngest fully expanded leaf (mm); H is the pseudo-stem height (mm); PPFD is the mean monthly photosynthetic photon flux density (mol m^{-2} mo^{-1}); ψ_{lp} is mean monthly predawn leaf water potential (MPa); and T is the mean monthly air temperature (°C).

The negative parameters of the PPFD variables for predicting leaf length (L) and pseudo-stem height (H) represent the etiolation caused by shade.

Anatomy

Cross-Section

Measured anatomical parameters differed between leaves grown in full sunlight and fluctuating light regimes (Table 3). There was an interaction ($p < 0.05$) between treatments and time as expressed by seasonal fluctuations. Thus, the leaf anatomical variables were greatest in spring (November 1999–2000) but reduced in summer (February 1999–2000) by water stress (mean ψ_{lp} of −1.3 MPa, −13.0 bar) and by air temperatures (10.2°C) in autumn (April 2000 and May 2001).

Leaves grown in full sunlight conditions were thicker ($p < 0.05$) than leaves grown under shade in all seasons. Epidermis of plants grown in full sunlight (O treatment) was only thicker ($p < 0.05$) compared with plants grown under ~24% transmissivity. Shading also induced a reduction ($p < 0.05$) in the number of cells across the cross-section and the number of mesophyll cells per unit area cross-section in full sunlight leaves was higher ($p < 0.05$) than in shaded leaves. Palisade cells decreased ($p < 0.05$) in height with shading to a greater degree than the reduction of palisade cell width. Plants grown under any shade regime had a smaller ($p < 0.05$) spongy diameter than plants grown in full sunlight conditions. The area of big and small bundles and the midrib bundle in the cross-section of the midpoint of the lamina were also reduced ($p < 0.05$) by shade. Also, the number of bundles per length cross-section was reduced ($p < 0.05$) to half for plants grown at ~24% transmissivity compared with full sunlight conditions.

Longitudinal Abaxial Surface

As for the cross-section analysis, the longitudinal abaxial parameters showed an interaction ($p < 0.05$) between treatments and time as expressed by seasonal fluctuations (Table 3). Shade increased ($p < 0.05$) epidermal cell length. For example, cell length for plants grown at ~24% transmissivity (T+S treatment) was doubled in spring and 30% longer in summer than those grown in full sunlight. In contrast, there were no differences ($p = 0.06$) in epidermal cell width between treatments. Epidermal cell elongation varied with reduced

Table 3. Anatomical parameters for the cross-section and longitudinal abaxial surface of orchardgrass leaves grown in four different light regimes (O = open 100% transmissivity, O+S = open plus slat shade ~43% of open PPFD, T = tree shade ~58% of open PPFD, T+S = tree plus slat shade ~24% of open PPFD). Data represent mean values of measurements taken on six occasions during 2 yr: spring (November 1999 and 2000), summer (February 1999 and 2000), and autumn (April 2000 and May 2001).

Seasons	Spring					Summer					Autumn				
Light intensity	O	O+S	T	T+S	SEM	O	O+S	T	T+S	SEM	O	O+S	T	T+S	SEM
Cross-section															
Leaf thickness, μm	169	148	150	119	6.30	156	133	140	109	9.54	160	139	142	112	6.40
Epidermis thickness, μm	24	22	23	19	1.10	21	20	20	18	0.57	23	21	21	18	0.60
No. mesophyll cells per area cross-section, No. $10^4 \times \mu m^{-2}$	13	16	17	21	1.44	15	19	20	25	1.19	14	18	19	23	0.29
No. of cell files in the cross-section	5.3	4.3	4.4	4.0	0.033	5.0	4.2	4.2	3.9	0.085	5.1	4.2	4.3	4.0	0.162
Cell palisade length, μm	38	30	31	28	0.29	31	28	28	23	0.72	32	28	28	24	0.33
Cell palisade width, μm	23	19	19	18	1.25	19	17	17	15	0.28	21	19	18	16	0.57
Cell spongy diam., μm	28	23	24	19	1.34	24	22	23	17	0.33	27	24	24	18	0.28
No. bundles per length cross-section	33	23	24	18	1.70	28	19	21	15	1.09	30	22	23	16	1.44
Area bundles, $10^2 \times \mu m^{-2}$															
Midrib	42	35	38	30	1.65	35	26	29	24	1.28	41	33	35	28	2.05
Big	36	31	34	25	2.23	31	22	24	20	1.37	35	26	28	23	1.37
Small	7	6	5	4	0.55	6	5	5	4	0.54	7	6	5	4	0.43
Longitudinal abaxial surface															
Leaf area, mm^2	540	591	565	620	16.7	170	235	156	178	7.26	250	279	242	294	10.8
Length epidermal cell, μm	235	380	417	462	14.6	195	263	244	278	13.7	207	283	245	318	10.2
Width epidermal cell, μm	35	32	33	30	1.05	32	30	31	28	1.00	33	31	33	29	1.05
No. epidermal cells per leaf abaxial area, $\times 10^2$	596	540	541	529	12.4	423	413	395	388	9.2	507	473	453	458	4.5

light intensity, water stress, and low temperatures to a greater degree than the reduction of cell number per leaf area. This was confirmed by the strong linear relationship ($R^2 = 0.84$) between epidermal cell length and lamina length (Fig. 4).

Leaf Photosynthesis

The light regimes caused a pronounced effect on the parameters of net photosynthesis as a function of PPFD (Fig. 5). The mean values of leaf photosynthesis parameters obtained from light curves when temperature, water status, and N were nonlimiting are shown in Table 4. The maximum P_{max} value was 27.4 μmol CO_2 m^{-2} s^{-1} saturated at 1100 μmol m^{-2} s^{-1} PPFD for plants grown in full sunlight. Under fluctuating light regime conditions both P_{max} and the saturation point decreased. When measured in full sun conditions (1800–1900 μmol m^{-2} s^{-1} PPFD) plants grown under ~24% transmissivity (T+S treatment) had lower ($p < 0.05$) P_{max} and saturation point than plants grown in the full sunlight (O) treatment or under tree shade (~58% transmissivity). However, when these plants were subsequently exposed to severe shade (129–130 μmol m^{-2} s^{-1} PPFD), T+S treatment had the highest ($p < 0.05$) P_{max} and saturated at the minimum PPFD value compared with O+S and T treatments. There was no difference ($p = 0.39$) between treatments in α when plants were in full sunlight, but

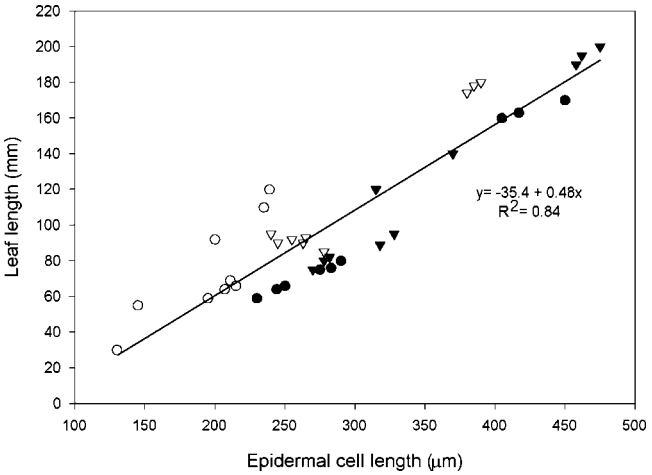

Fig. 4. Relationship between length of the youngest fully expanded leaf and epidermal cell length in the longitudinal abaxial surface of the youngest expanded leaf grown at 100% (open circle), ~43% (open triangle), ~58% (closed circle), and ~24% (closed triangle) transmissivity.

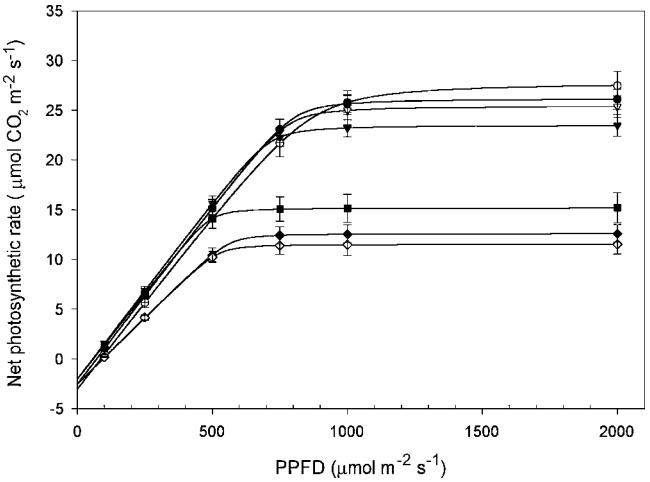

Fig. 5. Net photosynthesis rate against light intensity (photosynthetic photon flux density, PPFD) for orchardgrass leaves grown in a field environment at full sun in the open (open circle); in sun conditions (1800–1900 μmol m^{-2} s^{-1} PPFD) for plants grown under the slat structure in open (O+S) (open triangle), under trees (T) (closed circle), and under the slat structure plus trees (T+S) (closed triangle); and under severe shade conditions (120–130 μmol m^{-2} s^{-1} PPFD) for plants in the O+S (closed diamond), T (open diamond), and T+S (closed square). Bars indicate standard error of the mean (SEM).

Table 4. Light-saturated rate of net photosynthesis (P_{max}), initial slope (α), degree of curvature (θ), saturation point, and dark respiration (at 0 PPFD) of the orchardgrass leaf light-response curve, and stomatal conductance at saturation point for four light regimes (O = open 100% transmissivity, O+S = open plus slat shade ~43% of open PPFD, T = tree shade ~58% of open PPFD, T+S = tree plus slat shade ~24% of open PPFD). Measurements were taken when water, N content, and temperature were not limiting.

	O	O+S†		T†		T+S†		
Variables	Full sun	Shade	Sun	Shade	Sun	Shade	Sun	SEM
P_{max}, μmol CO_2 m^{-2} s^{-1}	27.4	12.6	25.4	11.5	26.1	15.1	23.5	0.41
α, μmol CO_2 μmol^{-1} PPFD	0.036	0.027	0.035	0.026	0.035	0.032	0.034	0.0008
θ, dimensionless	0.94	0.98	0.97	0.98	0.97	0.99	0.98	0.004
gs, mol H_2O m^{-2} s^{-1}	0.45	0.30	0.39	0.29	0.41	0.28	0.37	0.017
Saturation point, μmol PPFD	1100	600	900	600	900	500	750	11.9
Dark respiration, μmol CO_2 m^{-2} s^{-1}	−3.0	−2.5	−2.7	−2.6	−2.8	−2.0	−2.2	0.09

† For these fluctuating light regimes, photosynthesis measurements were taken after 120 min of severe shade (5–7% of the open PPFD) and after 45 min in full sunlight when induction was completed.

values of α decreased ($p < 0.05$) under shade. Plants grown under ~24% transmissivity had the highest ($p < 0.05$) α value compared with other shaded treatments. Plants grown under any shade regime had higher θ values than plants grown in full sunlight either in sun or exposed to severe shade. Under shade conditions, dark respiration was lower ($p < 0.05$) than the full sunlight treatment. Compared with full sunlight conditions, gs decreased ($p < 0.05$) under severe shade conditions and was lowest in the T+S treatment.

Adaptations Related to Leaf Photosynthesis

The mean canopy leaf angle of plants grown under ~24% transmissivity (T+S treatment) was 9° lower ($p < 0.05$) than orchardgrass pastures in full sunlight (Table 5). While foliage N content was similar ($p = 0.43$) between treatments, total chlorophyll content increased ($p < 0.05$) and the chlorophyll *a*/*b* ratio decreased ($p < 0.05$) with decreasing light intensity. The N/Chl ratio of plants grown in full sunlight (100% transmissivity) and under tree shade (~58% transmissivity) were significantly ($p < 0.05$) greater than ratios in plants grown under ~24% transmissivity. The A_{mes}/A ratio for plants grown in full sunlight was higher ($p < 0.05$) than the shaded treatments. The stomatal frequency on the abaxial surface of the youngest expanded leaf was higher ($p < 0.05$) for plants grown in full sunlight compared with plants grown under fluctuating light regimes but there were no differences ($p = 0.12$) in stomata length between treatments. This resulted in a greater ($p < 0.001$) total pore space in leaves grown in full sunlight than under shade.

Table 5. Main leaf adaptation related to leaf photosynthesis of orchardgrass leaves grown in four different light regimes (O = open 100% transmissivity, O+S = open plus slat shade ~43% of open PPFD, T = tree shade ~58% of open PPFD, T+S = tree plus slat shade ~24% of open PPFD). Measurements of mean leaf angle, specific leaf area (SLA), chlorophyll, leaf N content (N), leaf N/chlorophyll content ratio (N/Chl), mesophyll surface area/leaf surface area ratio (A_{mes}/A), stomatal frequency per mm^2 (f) of abaxial leaf area, the mean length of individual stomata (L), and total pore space ($f \times L$) were taken when water, N content, and temperature were not limiting.

Variables	O	O+S	T	T+S	SEM
Mean leaf angle, degrees	68	64	65	59	1.02
SLA, m^2 kg^{-1} DM	27	34	33	38	1.33
Chlorophyll *a*, g m^{-2}	0.52	0.59	0.58	0.61	0.014
Chlorophyll *b*, g m^{-2}	0.15	0.21	0.20	0.24	0.011
Chlorophyll *a*/*b* ratio, dimensionless	3.5	2.8	2.9	2.5	0.12
N, g N kg^{-1} DM	40	42	41	43	1.24
N/Chl, mg mg^{-1}	2.2	1.5	1.6	1.3	0.09
A_{mes}/A, dimensionless	23	18	18	15	1.01
Stomata frequency *f*, No. mm^{-2}	175	123	128	110	10.54
Stomata length L, μm	40	38	39	36	1.12
Total pore space $f \times L$, μm mm^{-2}	7000	4675	4992	3960	255.5

DISCUSSION

Morphology

The wide range of leaf dimensions over seasons emphasized the orchardgrass leaf plasticity under different light and environmental regimes. Shade induced an elongation of leaves and pseudo-stem (Fig. 3), particularly in spring when water and temperature were largely nonlimiting (September–November). The ~30% increase in length of the youngest fully expanded leaf and pseudostem of these orchardgrass pastures under shade of ~24% transmissivity (T+S treatment) was similar to that found by Schnyder and Nelson (1989) for tall fescue grown in low light conditions (60 μmol m^{-2} s^{-1} PPFD). Based on Eq. [2] and [4], 80% of the etiolation in leaf length and pseudo-stem height was caused by the light reduction as a response of grasses to compete for available light. The ability to etiolate was 70% lower (Eq. [2] and [3]) when soil volumetric water content was <15%, and 30% lower when temperatures were <11°C. Etiolation of shaded orchardgrass plants also occurred in response to a reduction in the R/FR ratio. Differential absorption of red and far-red light from the tree canopies determined that the R/FR decreased by 56% in the middle of the tree shade compared with full sunlight (Table 2).

Anatomy

As expected, higher light intensity produced thicker leaves (Chabot and Chabot, 1977; Sims and Pearcy, 1992) associated predominantly with an increase in mesophyll cells size (bigger spongy and higher palisade cells) and to a lesser extent by the number of cells (Table 3). This was consistent with Charles-Edwards et al. (1974), who reported that the increase in leaf thickness of three perennial ryegrass populations with increasing light intensity from 60 to 250 W m^{-2} was predominantly associated with increased mesophyll cell size. In the abaxial

surface, light intensity had little effect on the rate of cell division in the intercalary meristem from which leaves develop compared with cell elongation. Consequently, the elongation of orchardgrass leaf length was related primarily to reductions in epidermal cell length (Fig. 4) rather than a reduction in cell number. Forde (1966) also found a significant linear regression of lamina length and epidermal cell length for orchardgrass under different continuous shade intensities, but the slope of this regression was higher (0.63) compared with the slope of the present study (0.48) for fluctuating light regimes. Thus, a unit change in cell length of orchardgrass plants grown under continuous shade is associated with a greater change in lamina length than occurs in plants grown in fluctuating light regimes.

Water stress during the summer period (mean ψ_{lp} of −1.3 MPa, −13.0 bar) predominantly reduced the cell elongation of the epidermis and cell size of the mesophyll, and to a less extent the number of cells per leaf abaxial area and per cross-section for the four light regimes (Table 3). Also the area of bundles was reduced during the summer drought for all treatments. Thus, water stress and low temperatures affected leaf anatomy independently of the light intensity. Utrillas and Alegre (1997) reported that water stressed leaves (ψ_{lp} of −0.86 MPa, −8.6 bar) of bermudagrass [*Cynodon dactylon* (L.) Pers.] grass reduced both mesophyll and bundle sheath cell areas by 25 and 16%, respectively, compared with well-irrigated plants as an adaptation to reduce water loss and therefore maintain high tissue water potential. Meristems are considered to maintain cell division, even under water stress that stops cell expansion (Lawlor and Leach, 1985) and this differential sensitivity agrees with the result found in the present study. The reason for this greater sensitivity seems to be that cell expansion is largely a physical process driven by the hydrostatic pressure or turgor pressure within cells (Jones, 1988).

Similarly, low temperatures (mean air temperatures <11°C) in autumn reduced cell elongation of the epidermis and cell size of the mesophyll and, to a lesser extent, cell number for the four light regimes (Table 3). This agrees with Cooper and Edwards (1964), who reported that the greater final length of leaves of temperate grasses grown in optimum temperatures (20–25°C) was due mainly to a greater cell length rather than to an increase in cell number. Together these results imply cell division was a more conservative process than cell expansion under differing light regimes and environmental conditions.

Adaptations Related to Leaf Photosynthesis

Mean Leaf Angle

Under severe shade (T+S treatment), the mean canopy leaf angle was 9° more horizontal than orchardgrass pastures grown in full sun conditions (Table 5). This response would increase the capture of radiation under the severe shade situations and thus maximize individual leaf photosynthetic input (Charles-Edwards, 1981). However, during periods of full sun in the fluctuating light regimes the lower leaf angle is a disadvantage because most of the light is intercepted at the top of the canopy and does not penetrate deep into the canopy. As a consequence, canopy photosynthesis will be compromised, particularly at a LAI < 3 (Peri, 2002).

The reduced mean leaf angle under severe shade was caused by longer (Fig. 3A) and thinner leaves (Table 3). This was consistent with Deckmyn et al. (2000), who reported that orchardgrass leaves drooped from 68.7° to 53.9° as their length increased. Also, the more horizontal leaves of shaded plants was a consequence of a lower number and smaller size of bundles (Table 3), which provided a less rigid structure to leaves. Similar results have been reported for perennial ryegrass (Evans, 1964) although the magnitude of these leaf adaptations under continuous shade were lower compared with the current results founds for orchardgrass in fluctuating light regimes of similar transmissivity.

Specific Leaf Area (SLA)

As a consequence of the anatomical (small cell and bundle sizes under shade) and morphological (leaves being longer, narrower, and thinner under shade) adaptations to different light regimes, orchardgrass plants increased the SLA with decreasing light intensity. Similarly, Evans (1996) reported that the increase in SLA (or decrease in specific leaf weight, SLW) with shade for several species was caused mainly by an increase in mesophyll cell size, larger vascular bundles and sclerenchyma tissue, and thicker epidermal layers. The increased SLA of shaded orchardgrass may maximize light interception providing a greater surface for light adsorption for photosynthesis at the expense of leaf thickness (Table 3). However, Eagles (1973) reported an increase of 70% in SLA of a Norwegian orchardgrass population grown in a 80% light reduced environment, which is greater than the result found in the present study. This was because Eagles (1973) used a continuous rather than a fluctuating light regime.

Leaf Photosynthesis

There were three main features found in this study that showed leaf adaptations to the different light regimes that influenced leaf photosynthesis. These were: (i) P_{max} (and to a lesser extent α) in full sun conditions (1800–1900 μmol m^{-2} s^{-1} PPFD) was doubled and θ was lower than under shade (129–130 μmol m^{-2} s^{-1} PPFD); (ii) in full sun conditions plants previously grown under shade were photosynthetically less efficient than plants previously grown in full sunlight with lower P_{max} and α values (Table 4); (iii) when plants were exposed to shade, leaves adapted to severe shade condition (T+S treatment) had the highest P_{max}, α, and θ, and saturated at a minimum PPFD value compared with the O+S and T treatments.

First, a reduction in gs was a factor that would have decreased P_{max} under severe shade (Table 4). Sharkey and Ogawa (1987) reported that stomata can respond to light absorbed by pigments within the guard cell, indirectly as a response to the decrease in CO_2 in inter-

cellular spaces due to an increase of photosynthesis, and by responding to some agent from the mesophyll cells to the guard cells. In the present study, the reduction in gs was reflected in a reduced stomatal density (total pore space), which decreased with decreasing light intensity (Table 5) due to a decrease in stomata frequency rather than stomata length. Similarly peach leaves grown at a continuous light intensity of 25% PPFD had reduced stomata density (Nii and Kuroiwa, 1988) but the magnitude of response was lower than for the orchardgrass grown under the fluctuating light regime of similar light intensity.

The reduction in leaf photosynthesis in fluctuating light regimes resulted from a reduction of gs and also nonstomatal limitations (Pearcy, 1988; Peri et al., 2002b). Peri et al. (2002b) reported that the decrease in P_{max} for orchardgrass leaves exposed to 120 min of severe shade (5% of the open PPFD) was due to 52% nonstomatal and 48% stomatal limitations. In the present study, the reduction in α for plants under severe shade provides evidence of a nonstomatal limitation. This was confirmed by Peri et al. (2005), who reported that values of α for orchardgrass plants from high to low light intensities decreased up to 20% as a function of the magnitude and duration of the PPFD level previously experienced.

The differential responses in leaf photosynthesis would also be due to the shifting N investment in carboxylation, bioenergetics, and light harvesting along light gradients. In the present study, plants adapted to shade invested leaf N to chlorophyll, expressed as a decrease in the N/Chl ratio (Table 5), with decreasing light intensity. This agrees with Evans (1996) who reported that leaves grown under high irradiances had a considerable decrease in the chlorophyll/N ratio across a broad range of species and both herbaceous and tree species. Also, there was a decrease in the chlorophyll *a*/*b* ratio with a decrease in light intensity (Table 5), which is a common adaptation to enhance absorption of the limited red light under forest shade and to maintain the energy balance between photosystems PSII and PSI (Boardman, 1977; Evans, 1996). This may improve the light absorptance per unit N invested in light harvesting at low light levels. These changes provide explanations for the higher values in P_{max} and α of leaves adapted to severe shade condition (T+S treatment) compared with the O+S and T treatments when plants were exposed to shade (129–130 μmol m^{-2} s^{-1} PPFD). Niinemets and Tenhunen (1997) also found that sugar maple (*Acer saccharum* Marsh.) plants assimilated CO_2 at a several times higher rate than the leaves grown at 932 μmol m^{-2} s^{-1} PPFD when exposed to low light intensity due mainly to a greater N investment in light harvesting. They reported that the proportion of leaf N in Rubisco and in bioenergetics had hyperbolic positive response to increase light intensity through increments in leaf dry mass per area (or decrease SLA). Because in the present experiment SLA decreased and plants invested less N to chlorophyll with increasing light intensity (Table 5), it is likely that the partitioning of leaf N in Rubisco increased in leaves grown in full sunlight and this may determined the higher P_{max} and α values of sun plants in full sunlight conditions (Table 4). These partitioning adaptations also explain the higher values of θ for shaded plants (Table 4). Ögren (1993) reported that values of θ for willow (*Salix* sp.) leaves increased with shade due to their lower capacity of Rubisco relative to that of electron transport.

The acclimation to light regimes was also characterized by adjustment in leaf anatomy (mesophyll cell size and number). In general, P_{max} and α of orchardgrass leaves in sun conditions (Table 4) was related inversely to the SLA (Table 5) and directly to leaf thickness (Table 3). The decrease in leaf thickness with decreasing light availability was expressed as a reduction in the A_{mes}/A ratio from 23 (at 100% transmissivity) to 15 (at ~24% transmissivity) (Table 5). The greater A_{mes}/A ratio in sun plants can also explain their higher P_{max} in full sunlight conditions compared with the shade adapted plants. Chabot and Chabot (1977) reported that the A_{mes}/A ratio explained 61% of the net photosynthesis rate per unit area response of strawberry (*Fragaria vesca* L.) plants to different continuous light intensities. Greater A_{mes}/A ratio meant an increase in diffusion pathlength in sun plants. Many authors have argued that CO_2 diffusion from the intercellular airspaces to the sites of carboxylation may limit the rate of CO_2 assimilation (Evans, 1996). Therefore, to achieve higher rates of CO_2 assimilation in an environment with higher irradiance would require a larger surface area exposed to intercellular airspace per unit leaf area, which is represented by a greater A_{mes}/A ratio. The increase rate of CO_2 assimilation therefore requires an increased conductance of CO_2 through the leaf surface. In the present study, this was achieved by increased stomatal conductance and by greater stomatal density for sun plants (Table 5). In addition, in the present study, the more columnar palisade cells in the open-grown leaves (Table 3) would allow the directional light to penetrate further into the leaves increasing, therefore, the light absorbed by the chloroplast (Cao, 2000).

Finally, orchardgrass plants adapted to ~24% transmissivity had the lowest maintenance respiration and saturated at lower PPFD, which increase the efficiency of photosynthesis in low light conditions compared with other shaded treatments (Table 5). This agrees with the review of Boardman (1977) and Givnish (1988) for several species.

Implications for Orchardgrass Silvopastoral Pastures

There was an interrelationship between morphological and physiological leaf adaptation to fluctuating light regimes, which is a specific component of silvopastoral systems. Leaf adaptations can modify DM accumulation by affecting light interception and the photosynthetic activity of individual leaves. Specifically, results from the present study showed that the fluctuating light regimes decreased the parameters of P_{max}, α, and maintenance respiration and increased the parameter θ of orchardgrass plants compared with those grown in full sunlight conditions. These responses can be used to assist the pre-

diction of pasture DM growth through their incorporation into a canopy photosynthesis model for silvopastoral systems (e.g., Peri et al., 2006). Currently, this model does not account for adaptation of leaves to light regimes. Changes in these physiological variables related to anatomical adaptations under fluctuating light regimes can provide a theoretically explanation of a proportion of the variation in DM growth found in silvopastoral systems.

Also, results found in the present study related to morphology, mean leaf angle, and SLA of orchardgrass pastures grown under fluctuating light regimes can alter the canopy architecture, which determines the distribution of irradiance over the photosynthetic surfaces and hence, the possibility for high canopy radiation use efficiency (RUE). This becomes important in silvopastoral systems where low irradiance is imposed by the tree shade and can be used for calibrating models which utilize RUE to predict DM production.

Prediction of pasture production on a farm basis is an important part of feed planning. Feed profiling (for appropriate stocking rate), feed budgets (for seasonal planning), and grazing plans (short-term planning to achieve desired intakes and rotation length) need an accurate assessment of DM production. Using the canopy photosynthesis model adjusted with parameters found in the present study different seasonal scenarios affecting DM production (e.g., dry summer under fluctuating light regime), may provide different strategies for farmers. Also, it is possible to simulate the potential increase in DM production (or the equivalent of animal performance) from N fertilizer or irrigation interacting with shade in silvopastoral systems.

CONCLUSIONS

This study quantified morphological, anatomical and physiological adaptations of orchardgrass plants grown under fluctuating light regimes and integrated with other environmental factors specific to silvopastoral systems. In silvopastoral systems, understorey plants experience frequent fluctuations in irradiance from full sun to shade caused by tree canopy shading. This study has shown several adaptations that operate during such fluctuations were different from those that operate under steady-state conditions. In particular, compared with results from steady state shade plants under fluctuating regimes showed a lower change in lamina length per unit in epidermal cell length, a higher number and size of bundles, which provided rigid structure to leaves, a lower increase in SLA, a higher reduction in stomatal density (total pore space) that reduced gs with decreasing light intensity, and new parameters of P_{max}, α, θ, and maintenance respiration for orchardgrass plants during the induction process (recovery of leaf photosynthesis from low to high irradiance). Thus, to represent understorey responses of species in silvopastoral systems or other fluctuating light regime condition, slatted rather than cloth structures are required. The photosynthetic responses and adaptations of orchardgrass leaves to fluctuating light regimes can be used for inclusion in a canopy photosynthesis model of silvopastoral systems for prediction of pasture DM growth.

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