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Diurnal variation of photosynthesis and photoinhibition in tea: effects of irradiance and nitrogen supply during growth in the field

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Abstract

Diurnal changes in the rate of photosynthesis (*A*) of mature tea (*Camellia sinensis* (L.) O. Kuntze) bushes grown at high elevation in the field in Sri Lanka, were related to environmental conditions. Bushes were either unshaded, receiving 100% of incident photosynthetically active radiation (PAR), moderately shaded, (65% PAR) or heavily shaded (30% PAR). These treatments were combined with nitrogen fertilizer applications of 0, 360 and 720 kg ha^{-1} year^{-1}. When recently fully expanded leaves were measured under the growing conditions on bright, clear days from dawn to dusk, *A* was greatest in the morning with increasing radiation between approximately 8 h and 10 h. Stomatal conductances (g_s) and substomatal carbon dioxide concentrations (C_i) were then large, leaf temperatures (T_L) cool, and saturated water vapour deficits (*VPD*) small. However, as the irradiance, T_L and *VPD* increased towards midday, *A*, g_s, photochemical quenching, and C_i decreased, and non-photochemical quenching increased. In the late afternoon, irradiance, T_L and *VPD* fell, but despite the relatively large increase in g_s and C_i, *A* remained low; however, it recovered overnight. The zero-N treatment decreased total-N content of leaves by 50% and *A* by *c*. 20% (not significant). Leaves of unshaded plants receiving least N had significantly ($P < 0.05$) smaller *A* and greater total sugar content than shaded but with abundant N, *A* and sugars did not differ between shade treatments. Analysis of the responses of *A* to environment in the morning compared to the afternoon, and of chlorophyll fluorescence, suggests that *A* was photoinhibited as a consequence of greatly increased PAR, whilst decreasing g_s (related to changes in PAR, *VPD* and T_L) caused C_i to fall. End-product inhibition of *A* is not consistent with decreased C_i. Inhibition of *A* as a result of photoinhibition was minimized, but not eliminated, by abundant N. Interactions between factors regulating *A* in tea are discussed.

Key words: *Camellia sinensis* (L.) O. Kuntze, chlorophyll *a* fluorescence, nitrogen, photoinhibition, photosynthesis, solar radiation, tea.

Introduction

Tea (*Camellia sinensis* (L.) O. Kuntze) is a small, evergreen, woody perennial tree, cultivated for the production of leaves, which are manufactured into a beverage. Total biomass and dry matter yields of harvested (plucked) young shoots are relatively small (500–2500 kg ha^{-1} year^{-1}) because, in part, harvesting removes much of the active productive area, nutrients etc. To understand the regulation of production in the crop, it is necessary to consider what determines dry matter production (Hadfield, 1968; Carr, 1972; Carr and Stephens, 1992). This depends on the total net accumulation of assimilated carbon (i.e. photosynthesis minus respiration), and the relatively small accumulation of mineral nutrients, by the crop over a period. Dry matter accumulation depends on the photosynthetic rate per unit leaf area (A) and on the formation of crop leaf area which depend on the potential for formation of leaf buds and on the availability of assimilates for their growth. Rates of A are determined by the characteristics of the

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photosynthetic machinery, including the capacity, which are not fixed but change with environmental conditions during growth. Also, A depends strongly on environmental conditions, such as irradiance, temperature and nutrient supply, so that A changes diurnally and seasonally. Thus, productivity is a complex function of plant characteristics and environment (Lawlor, 2001).

Low productivity of tea may be related to inadequate assimilate production ('source limitation'), as the rates of photosynthesis (A) of its leaves are small compared to many other tropical plants, being in the range of 2–14 μmol m^{-2} s^{-1} (Squire, 1977; Roberts and Keys, 1978; Smith *et al*., 1993). However, it is comparable to coffee (*Coffea arabica*; Nunes *et al*., 1993; Ramalho *et al*., 1997), and other trees (Wullschleger, 1993). Photosynthesis of tea has received less attention than other physiological processes likely to improve production. In part, this is due to the belief that tea has an inadequate number of growing shoots to use the assimilates produced by photosynthesis, i.e. it is 'sink limited' (Tanton, 1982; Squire, 1985; Rahman, 1988). Therefore, it has been argued (Tanton, 1992), that the improvement of photosynthesis would not benefit production, and the growth potential of the organs must first be improved.

However, there is evidence that photosynthesis of tea is decreased by strong solar radiation, so that shade might benefit production. Tea is a shade plant, adapted to the understorey of forests in its native habitat, and hence it is often grown under shade (which is relatively easy to apply via shade trees or screens), although there is much controversy about this agronomic practice (Obaga, 1984; Carr and Stephens, 1992). The intensity of radiation is regarded as important because of photoinhibition (PI) (Smith *et al*., 1993). PI decreases the capacity for A in many plants (Baker and Bowyer, 1994), including coffee, which has similar physiology to tea, and responds similarly to radiation and shade (Nunes *et al*., 1993; Ramalho *et al*., 1997). PI is caused by damage to the photosynthetic system in strong radiation as a consequence of excess energy and an over-reduced state of photosynthetic components. Excess energy is determined by the balance between energy captured and used in CO_2 assimilation and photorespiration or dissipated by the xanthophyll cycle (Baker and Bowyer, 1994; Lawlor, 2001). PI may be short-term and reversible or long-term and irreversible, and is related to increased leaf temperature and to large photon flux when electron transport to acceptors is limited (Melis, 1999). In general, the photosynthetic capacity of tea is greater for leaves grown in the shade than unshaded (Rahman, 1988): the causes and mechanism of this are unclear (Carr and Stephens, 1992). Cooler leaf temperatures and moister air correlate with decreased radiation, and may be responsible for increased A.

Leaf temperature is important in tea production, as it affects organ growth (Tanton, 1982), although A is less sensitive (Smith *et al*., 1993). Maximum A of tea occurs at leaf temperatures (T_L) between 25 °C and 30 °C under saturating light and with atmospheric CO_2 (Hadfield, 1976), and A decreases rapidly at T_L above 35 °C and ceases completely above 40 °C. The T_L is decreased by between 2 °C and 12 °C under shade, compared to bright sun, and is also affected by leaf angle, with horizontal leaves 2–4 °C warmer than erect leaves (Hadfield, 1968). However, shade not only decreases T_L but also increases humidity, which increases A, as small vapour pressure deficits (VPD) increase stomatal aperture (Carr, 1972). This increases conductance to CO_2 diffusion, so increasing CO_2 for photosynthesis. Dry conditions thus decrease yields (Tanton, 1982).

The photosynthetic capacity of leaves depends on the characteristics and amounts of the components of the photosynthetic machinery, the production of which depends on the availability of nutrients. Nitrogen is particularly important, as it is required for the synthesis of cellular components, including chlorophyll (and thus related to capture of PAR photon flux) and proteins such as Rubisco (responsible for CO_2 assimilation) so it is central to photosynthetic metabolism and growth (Lawlor, 2001). Hence, N deficiency decreases both source and sink capacity, by decreasing the formation of photosynthetic components (and thus A) and shortening the productive life-span of leaves. Also, the number and size of organs are decreased, limiting 'sink' capacity for the utilization of assimilates so that carbohydrates accumulate, and may lead to feedback inhibition of A and PI (Baker and Bowyer, 1994; Melis, 1999). Little is known of the relationship between N supply, leaf composition, photosynthetic capacity, and photosynthetic rates for tea in the field. Applying N fertilizers to tea crops suffering deficiency increased the N content of leaves and the yields of plucked tea significantly (Owuor *et al*., 1990). For this reason, N fertilizers form a significant part of the cost of tea production. Many plants suffer a proportionally larger inhibition of sink capacity than of source capacity as a result of N deficiency and thus are less able to use the radiation captured for CO_2 assimilation, and so may suffer from inhibition of photosynthesis, particularly under intense solar radiation (Baker and Bowyer, 1994). In coffee, adequate N-supply increased growth, photosynthesis and the content of photoprotective pigments, and decreased the damage caused by high-light (Nunes *et al*., 1993; Ramalho *et al*., 1997).

It was hypothesized, based on the evidence, that abundant N and shade will increase or maintain A and minimize photoinhibition in leaves of tea compared to deficient-N and full solar radiation. This hypothesis has been examined in mature, clonal tea growing in the field and subjected to different N fertilizer applications and to different shade treatments over a 9 month period. By measuring A, chlorophyll fluorescence and carbohydrates

and relating them to environmental conditions over the course of the day when environmental factors are changing, the influences of irradiance, temperature and *VPD* on photosynthesis were assessed. The long-term, practical aim of the work (Mohotti, 1998) was to describe and analyse the effects of different environmental factors on the photosynthesis of tea in order to guide the development of agronomic practices for increasing crop productivity and harvestable yield.

Materials and methods

The experimental treatments were applied in March 1996 to 34-year-old, mature tea bushes of clone TRI 2025 in field number 8 at the St Coombs Estate, Talawakele, Sri Lanka (latitude 6°55′ N, longitude 80°40′ E, altitude 1382 m above mean sea level). The bushes had been pruned in June 1994. For October and November 1996, during the period of measurement, monthly average rainfall was 191 and 172 mm and air temperature was 18.3 and 18.8 °C, respectively. Details of treatments, measurements etc have been given previously (Mohotti, 1998).

Treatments

Treatments consisted of all combinations of three irradiances and three N applications. Irradiance was altered by suspending black nylon netting horizontally 90 cm above the top of the crop canopy. The unshaded treatment received full solar radiation which, on clear days, reached a maximum of approximately 2000 μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR). Medium shade gave approximately 65%, and heavy shade 30%, of the incident PAR. The nitrogen treatments applied were (as urea with 42% N), in kg N ha^{-1} $year^{-1}$, 0 (no N), 360 (medium N, the recommended application for high yielding tea; Sivapalan and Kathiravetpillai, 1992) and 720 (high N) to give excess N. All plots received 120 kg P_2O_5 ha^{-1} $year^{-1}$. All fertilizers were given in five equal amounts at equal intervals throughout the year.

The experimental design was a 3×3 factorial in completely randomized blocks with three replicates. Each plot consisted of 28 bushes. To eliminate edge effects, eight bushes in the centre of each plot, at least 0.5 m from the edge, were used for measurements.

Measurements

All measurements were made on intact plants in the field.

Photosynthesis and transpiration: Water vapour and CO_2 exchange of the leaves were measured at the experimentally altered PAR at which the plants were growing and at ambient CO_2 concentration (approximately 360 μmol mol^{-1}), during October and November 1996. Measurements were made on four consecutive days (30 October to 2 November) which were bright and clear with no cloud and with similar radiation. Two similar, healthy, recently mature leaves on top of the canopy in each replicate treatment were measured for gas exchange and leaf temperature (T_L) using a portable, closed-circuit infrared gas analyser (IRGA, model LI-6200, Li-Cor Inc., Nebraska, USA) with a 250 cm^3 cuvette. PAR photon flux was measured with a quantum sensor (Li-Cor Inc., Nebraska, USA), and air temperature with a thermocouple; T_L in the chamber was calculated using an energy balance equation. The g_s was calculated using T_L, transpiration, chamber air temperature, and boundary layer conductance of the leaf, and also the intercellular CO_2 concentration (C_i) (according to von Caemmerer and Farquhar, 1981). All the measurements were made when the leaf was enclosed in the cuvette.

Fluorescence: Chlorophyll *a* fluorescence of photosystem II (PSII) was measured using a portable modulated fluorescence meter (model OS-100, OSLOG-PP Systems, Hitchin, Herts, UK), on 2 d in the morning up to maximum PAR, using similar leaves to those used for photosynthesis. Minimal fluorescence under steady-state photosynthesis in actinic light (F'_o) was estimated using F_o, F_m, F_s, and F'_m as described previously (Oxborough and Baker, 1997). The leaves were exposed to actinic light and when fluorescence reached a steady-state (F_s), a saturating light pulse was applied to obtain the maximal fluorescence under actinic light (F'_m). Photochemical (q_P) and non-photochemical (q_{NP}) quenching parameters under a range of different light intensities were calculated as follows; $q_P=(F'_m-F_s)/(F'_m-F'_o)$ and $q_{NP}=1-(F'_m-F'_o)/(F_m-F_o)$ (Schreiber *et al*., 1986). At about midday, leaves were darkened for *c*. 1 h (covered with cuvettes supplied by the manufacturer), and then F_o was measured with a modulated light of 350–700 nm wavelength and F_m (maximal fluorescence) was determined after a saturating light flash of 2 s duration and approximately 10 000 μmol m^{-2} s^{-1} intensity. The intrinsic efficiency of PSII when fully oxidized, F_v/F_m, was calculated from $F_v=F_m-F_o$.

Leaf composition: Similar leaves to those used for the photosynthesis measurements were harvested around midday for the analysis of N and the measurement of carbohydrates after gas exchange. Total nitrogen contents of the leaves were measured by the Kjeldahl method, using leaf material that had been dried overnight at 95 °C and powdered. Each sample was replicated twice. For total sugars, leaf discs of known area were cut into a solution containing 60% methanol, 25% chloroform and 15% distilled water and stored at −20 °C, before homogenization. Soluble and insoluble fractions were separated by centrifugation in glass centrifuge tubes. The pellet was kept for starch analysis. An equal volume of chloroform was added to the supernatant, shaken and separated into chloroform and aqueous methanol phases by centrifugation. The methanol–water fraction was removed and used for the total sugar content determination: 50 μl of the sample was added to 0.9 cm^3 of anthrone reagent containing 0.15% w/v anthrone in 75% sulphuric acid, mixed, and incubated in 40 °C for 20 min in a water bath. Each sample was replicated twice. Optical density was read at 630 nm in a spectrophotometer (model GBC UV/VIS 911A, GBC Scientific Equipment Pvt Ltd., Victoria, Australia) in disposable plastic cuvettes. A standard curve relating known sugar concentrations of glucose, fructose and sucrose, versus absorbance, was made and the total sugar in the samples was calculated.

Starch content of leaves was measured in the pellet by extraction with 10 cm^3 80% methanol to remove traces of sugar. The extract was then centrifuged at 5000 rpm for 30 min, and the supernatant was discarded. To the residue, 10 cm^3 distilled water was added, well mixed and heated in a water bath at 100 °C for 30 min. It was then centrifuged at 5000 rpm for 30 min, supernatant was collected in 10 cm^3 volumetric flasks and made to volume. Two drops of a mixture of iodine (I_2) and potassium iodide (KI) was added to the solution and the absorbance measured at 660 nm wavelength. A standard curve of absorbance versus known amounts of soluble starch (treated

as the leaf samples) was made and the starch concentrations in the samples were calculated.

Statistical analyses

The data were analysed using the Genstat for Windows statistical package (Genstat 5, release 4.1, Lawes Agricultural Trust, 1997) and Sigmaplot. Analysis of variance, for one and two-way interactions, was used to assess treatment differences and interactions. Statistical significance was taken at $P > 0.05$. Means are given with plus and minus one standard error. The relationships between A and PAR, leaf temperature and stomatal conductance were analysed by linear regressions for the morning and afternoon, i.e. before and after 12.00 h local time, respectively). For A, only values measured at PAR equal to or exceeding 500 μmol m^{-2} s^{-1} (the saturating PAR for A of control plants at ambient CO_2) were included to ensure comparability.

Results

Environmental conditions

In Fig. 1 the values for environmental conditions are averaged over the 4 d of measurement as the conditions were so similar. The sun rose around 07.00 h local time and the radiation increased rapidly, so that by 08.00 h the PAR incident on the canopy of the unshaded plants exceeded 500 μmol m^{-2} s^{-1}. By 09.00 h, PAR was 1000 μmol m^{-2} s^{-1}, peaking at 2000 μmol m^{-2} s^{-1} between 11.00 h and 13.00 h. The PAR decreased thereafter dropping below 500 μmol m^{-2} s^{-1} after 15.30 h (Fig. 1A). In the medium and heavily shaded plots, PAR exceeded 500 μmol m^{-2} s^{-1} at about 08.00 h and 09.30 h, respectively, and decreased below that value after 15.00 h. The T_L (Fig. 1B) increased along with the increase in PAR and air temperature (not shown) from about 17 °C in all treatments just after sunrise to about 25 °C by 09.00 h and 30–32 °C at midday. By 14.00 h, T_L had dropped to *c*. 25 °C, reaching 22 °C in the late afternoon, when the treatments did not differ. During the midday period (11.00–14.00 h), T_L in the medium and heavily shaded plots was *c*. 1.5 °C and 2.5 °C cooler than the unshaded plots, respectively (significant at $P < 0.05$). At equivalent irradiance in each treatment T_L was cooler in the morning than in the afternoon. At sunset, leaves in all treatments were 4–6 °C warmer than at dawn.

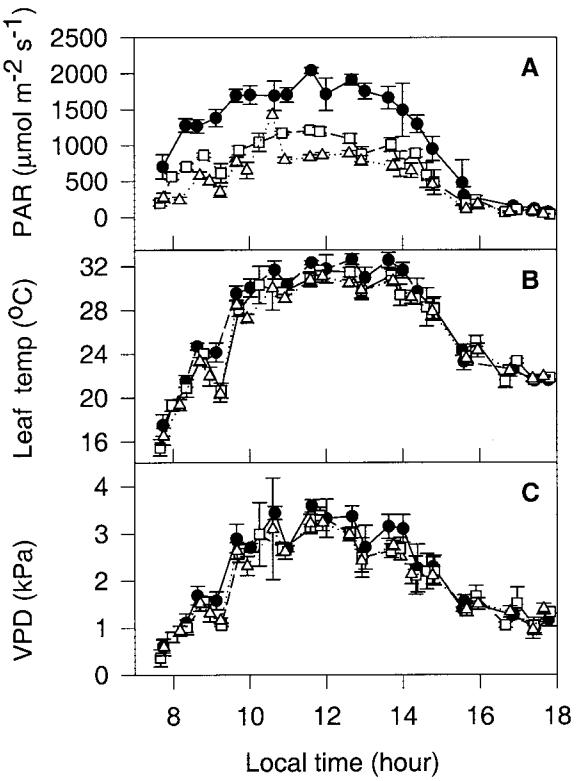

Fig. 1. Diurnal course of variation in photosynthetically active radiation (PAR) (A), leaf temperature (B) and vapour pressure deficit (*VPD*) (C) for tea crops grown without shade (●), or with medium shade (□) and heavy shade (△). Measurements were made on four, very similar consecutive days and the mean data are given. As there were no significant differences between N treatments the means of different N treatments are shown for each shade treatment. The vertical and horizontal bars indicate standard errors of means.

Vapour pressure deficit of the air (Fig. 1C) was about 0.5 kPa in the early morning, increasing to about 2.5 kPa at 10.00 h and reaching about 3.5 kPa at midday and by 15.00 h it decreased below 2 kPa, further decreasing to 1.5 kPa by sunset. The *VPD* was slightly (but not significantly) larger in the unshaded than shaded plots.

Photosynthesis

The average A of leaves for the 4 d of measurement for the different treatments (Fig. 2A–C) increased rapidly from zero at dawn (data not given) to a maximum between approximately 08.00 h and 09.00 h. This was followed by a progressive decrease, which was smaller and delayed in the shaded compared to unshaded treatments, especially at low N (Fig. 2B, C cf. A). In high N plots, there was no statistically significant difference in A between the shade treatments during the day, although in unshaded plots A tended to be slightly smaller than in shaded (Fig. 2A). Leaves of plants grown with medium N (Fig. 2B) without shade, had significantly lower A than the shaded plants after about 09.00 h. This was also apparent (but the decrease was smaller as a proportion of the shaded treatment) in plots receiving no N (Fig. 2C), where unshaded leaves had lower rates than shaded throughout the day. The decrease in A without shade after the early morning maximum to midday was statistically significant compared to shading in the medium and zero N treatments. Within each shade treatment, there was no significant difference ($P < 0.05$) in A between N treatments and no significant interactions between N and shade treatment at any time of day. When a repeated measures analysis was made of the effects of

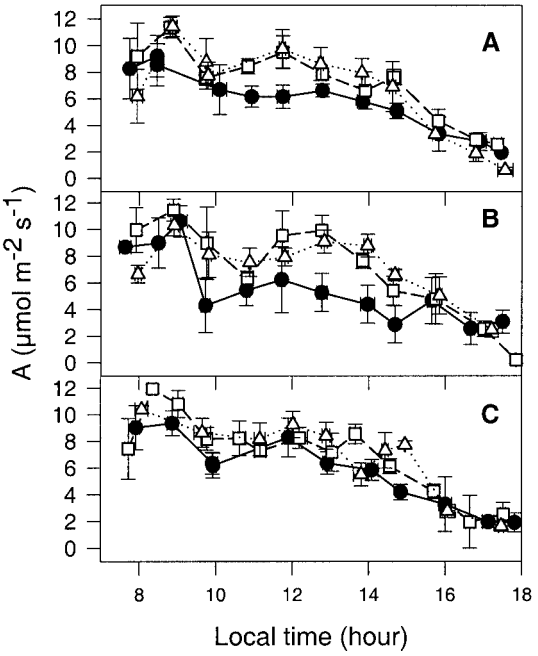

Fig. 2. Photosynthetic rates (A) of recently mature leaves of tea, in a field experiment with different shade and N supply. (A) No N applied, (B) medium N, (C) high N plants. The measurements were made at ambient CO_2 (360 μmol mol^{-1} CO_2) and under each irradiance at which the plants were grown. Vertical and horizontal bars indicate standard errors of each point which is the mean of measurements made on three leaves per treatment, on four different days (randomly selected leaves on each occasion). Symbols: plants grown without shade (●), medium shade (□) and heavy shade (△).

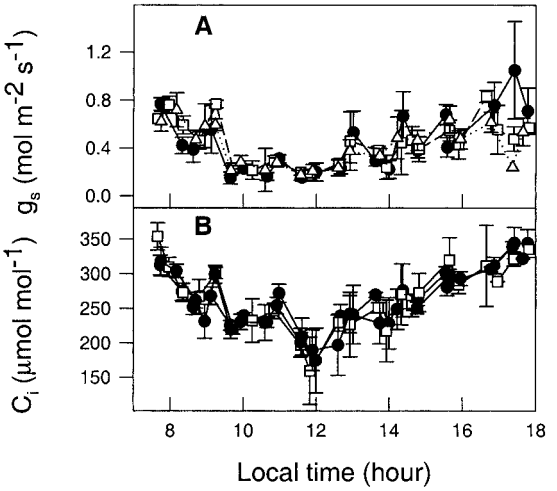

Fig. 3. Change in (A) stomatal conductance (g_s) and (B) intercellular CO_2 concentration (C_i) during the day, measured during photosynthesis measurements on leaves from a field experiment with plants grown without shade (●), or with medium shade (□) or heavy shade (△), averaged over N treatments. Each point is the mean of measurements on a total of nine different, recently mature leaves, taken at random on four different days. The vertical and horizontal bars indicate standard errors of means.

shade on A averaged over all treatments, the unshaded plots had significantly smaller rates than the shaded.

Stomatal conductance and internal CO_2 concentration of leaves

In the early morning g_s was maximal (Fig. 3A), it decreased towards midday and increased during the afternoon. There was no difference in g_s between the shade or N treatments. The C_i responded similarly to g_s (Fig. 3B): high in the early morning, reaching a minimum at about midday and increasing in the afternoon. There was no significant difference between treatments.

Fluorescence

The F_v/F_m ratio at about midday, was significantly smaller (when averaged over N treatments) in unshaded leaves (mean 0.64 ± 0.05) than shaded (mean of two shaded treatments 0.73 ± 0.04 which did not differ significantly). F_v/F_m was larger with abundant N than with low N application (0.73 ± 0.06 cf. 0.68 ± 0.06 mean for the two low N treatments).

Photochemical quenching decreased as a function of photon flux in all treatments (Fig. 4). It was significantly greater in the unshaded treatment than in the shaded

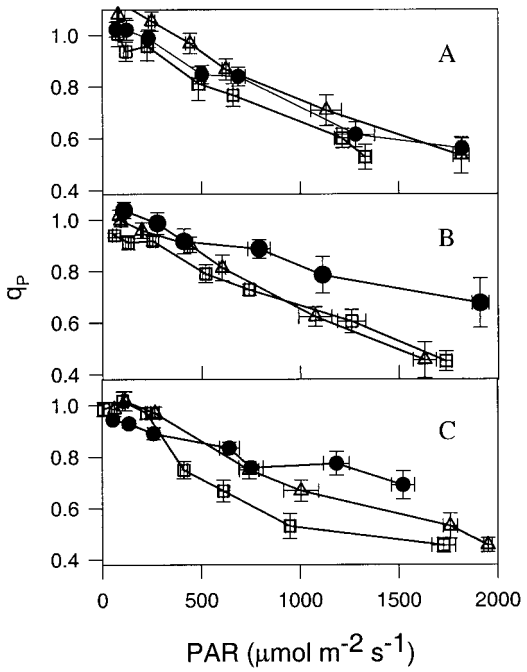

Fig. 4. Response of photochemical quenching (q_P) to varying photon flux in tea plants grown in the field without shade (●), with medium shade (□), or with heavy shade (△), combined with three different applications of N fertilizer: (A) no N, (B) medium N and (C) high N. Each point is the mean of a total of seven different leaves measured on four different days during the morning. Measurements were made at 360 μmol mol^{-1} CO_2. The vertical and horizontal bars indicate standard errors of means.

treatments with medium and high N-application (Fig. 4B, C, respectively), but not with low N. With medium N application, the two shade treatments did not differ, whereas with high N, q_P was significantly less in the medium shade treatment than the heavy shade. With medium and large N applications (Fig. 4B, C, respectively) q_P of the unshaded leaves was larger than with no N applied (Fig. 4A) as PAR increased.

Non-photochemical quenching increased with photon flux in all treatments (Fig. 5) and was smaller for leaves grown under shade in all treatments at low PAR flux and also smaller with medium and high N application (Fig. 5B, C) than with low N (Fig. 5A) at large PAR flux. Differences were significant between shade treatments at high N (Fig. 5C).

Leaf composition

The effects of conditions during growth on leaf N and carbohydrate contents were examined.

Leaf N content: Total N content per unit dry matter (N%) of leaves tended to decrease with limited N supply (from 3.1% to 1.5% over the range of N applications, data not shown). Those from heavy and medium shade had larger N content (average of 2.6%) than unshaded (2.29%), but there was no significant difference ($P<0.05$) in the effects of N applications or shade on N%.

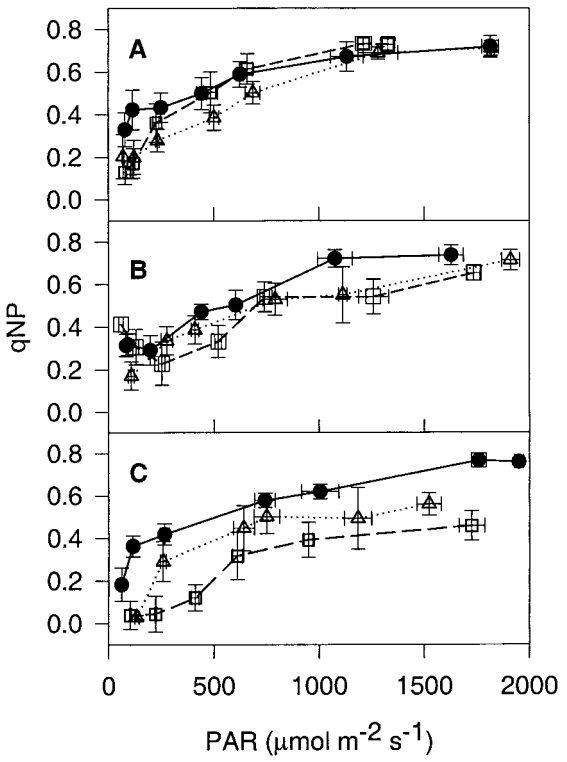

Fig. 5. Response of non-photochemical quenching (q_{NP}) to varying photon flux in tea plants grown in the field with three different applications of N fertilizer: (A) no N, (B) medium N and (C) high N plants. The data are for plants grown without shade (●), with medium shade (□) or with heavy shade (△). Each point is the mean of seven different recently matured leaves, randomly selected. Measurements were made at 360 μmol mol^{-1} CO_2 in the morning. The vertical and horizontal bars indicate standard errors of means.

Soluble sugar content: Increased N supply did not affect the amount of sugars per unit leaf area in the unshaded plants (mean 2.3 ± 0.06 g m^{-2} leaf) but increased it in shade (from 1.47 ± 0.09 to 1.87 ± 0.09 g m^{-2} leaf averaged over the two shade treatments). Unshaded plants had a greater sugar content (2.3 ± 0.15 g m^{-2}) than those in medium and heavy shade, which did not differ (mean of the two treatments 1.63 ± 0.12 g m^{-2}). The sugar content was smallest in the medium or no-N treatments, with shade.

Starch content

There was no significant difference ($P<0.05$) between treatments in starch content of leaves (1.2 ± 0.26 g m^{-2} averaged-over all the treatments), although the trend was for it to decrease with increasing N and shade.

Discussion

The treatments in this study (Mohotti, 1998) were designed to subject the mature tea crop to extremes in order to assess the response of photosynthesis to environmental conditions and to advance the understanding of the mechanisms by which photosynthetic rate is determined. However, the nitrogen treatments did not cause large effects, but shading did increase *A*. This agrees with other work (Barman *et al.*, 1993) and suggests that basic productivity of tea, in the conditions characteristic of the main tea growing areas of Sri Lanka, would benefit from shade.

The cause of increased *A* with shade is indicated by the analysis of the data. Regression of the maximum rate of *A* (measured at PAR above 500 μmol m^{-2} s^{-1} which saturates *A* measured from the light–response curves; data not presented) on PAR in the morning and afternoon, shows that leaves behaved very differently (Fig. 6A). In the morning, *A* was substantially greater (*c.* 30%) than in the afternoon at equivalent PAR (500–800 μmol m^{-2} s^{-1}, Fig. 6A). As the day progressed, irradiance increased to the large values characteristic of high solar elevations and altitude (*c.* 1300–1400 m above mean sea level). However, *A* decreased (Fig. 2A) and remained low, not returning to the morning rates as PAR decreased during the afternoon (Fig. 1A), although the PAR would have been sufficient. Thus, under these conditions, *A* decreased as a consequence of processes occurring during the morning. Inhibition was short-term, as *A* recovered in all treatments during the night to rates very similar to the previous day (data not given). The

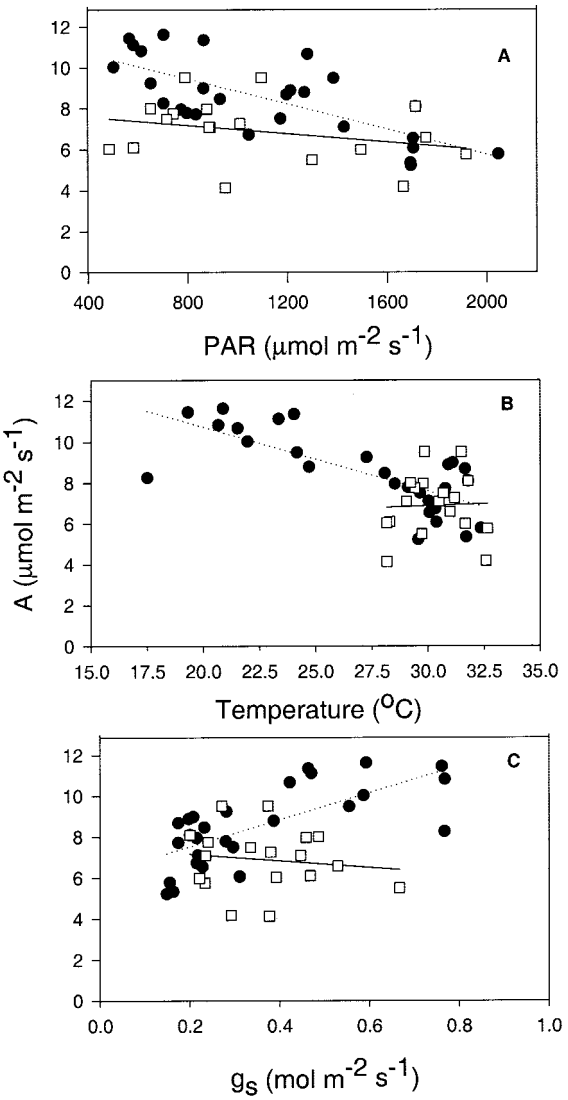

Fig. 6. The relationship between maximum rate of photosynthesis (A), measured at irradiances which saturate photosynthetic CO_2 assimilation (above 500 μmol m^{-2} s^{-1} PAR), and PAR (A), temperature (B) and stomatal conductance (C) in the morning (●, dotted line) or afternoon (□, solid line), i.e. before and after 12.00 h local time, respectively. (A) Rate of A related to PAR in the morning ($A = -0.0031\text{PAR} + 11.93$: $r^2 = 0.25$) and in the afternoon ($A = -0.0010\text{PAR} + 7.99$: $r^2 = 0.09$). Regressions significantly different between morning and afternoon. (B) Rate of A related to leaf temperature in the morning, ($A = -0.3126T_L + 16.98$: $r^2 = 0.54$) and afternoon ($A = -0.151T_L + 5.73$: $r^2 = 0.001$). Regressions significantly different between morning and afternoon. (C) Rate of A related to stomatal conductance (g_s) in the morning ($A = 6.62g_s + 6.18$: $r^2 = 0.49$) and in the afternoon ($A = -0.163g_s + 7.49$: $r^2 = 0.02$). Regressions significantly different between morning and afternoon.

decrease in A could be due to several factors, for example, large PAR flux causing PI, high temperatures inhibiting metabolism, or large *VPD* decreasing g_s and restricting the flux of CO_2 into the leaf, or decreased mesophyll conductance for CO_2 flux to the chloroplasts.

The relationship between A and PAR and T_L (Fig. 6A, B), suggests that as the day progressed the capacity for CO_2 assimilation was decreased by the high irradiance, or the associated increase in temperature. The large A and high q_P (Fig. 4) in the early morning decreased as PAR increased whereas the low initial q_{NP} increased. Shade maintained larger A and minimized the rise in q_{NP}. These changes show that as the PAR flux increased, energy captured was used less efficiently and more was dissipated, particularly in leaves fully exposed to light compared to those being shaded. As a consequence of the progressive increase in excess energy, leaves became photoinhibited during the course of the morning (indicated by the low F_v/F_m values) and were unable to recover during the later afternoon. These results resemble those of Ramalho *et al.* for coffee (Ramalho *et al.*, 1997). Leaves of coffee plants grown at PAR of 150 μmol m^{-2} s^{-1} were exposed (after 45 min at 400 μmol m^{-2} s^{-1}) to a PAR flux of 1500 μmol m^{-2} s^{-1}; after 3 h photosynthetic capacity was decreased by 40%, F_v/F_m decreased and q_{NP} increased. Both tea and coffee are species adapted to shade in the wild state, so sensitivity to large PAR flux is expected. The sensitivity of mature tea, which might be expected to adjust to large PAR flux (as coffee does; Ramalho *et al.*, 1997) is in agreement with other observations (Barman *et al.*, 1993), who reported from Tocklai, India, that A was 33% higher for shaded than unshaded tea plants. They attributed this to increased g_s and greater transpiration rate *per se*. However, transpiration is probably indirect, for example, related to cooler leaves. From the present analysis, the effect is likely to have been smaller PAR flux and thus less PI.

The decrease in A could be a consequence of the rising temperature during the morning and the continued high temperatures in the afternoon. At 20 °C in the morning, A was *c.* 37% greater than at 30 °C in the afternoon (Fig. 6A). Temperatures did not decrease substantially during the afternoon when PAR was saturating for A, and there was no recovery of A to early morning values. In general, A of all treatments decreased with increasing temperature; that of unshaded leaves in the low N plots showing the greatest proportional decrease. Hadfield considered that photosynthesis of tea in the field in India was optimal in the temperature range 25–37 °C and decreased when leaf temperature exceeded 37 °C (Hadfield, 1975). The present data show (Fig. 6B) that in the morning, light saturated A decreased progressively between *c.* 17 °C and 32 °C, so A may be more sensitive to T_L than previously thought. However, Smith *et al.* showed for a field experiment in the southern highlands of Tanzania, that A measured in the canopy was very constant between 20 °C and 32 °C (Smith *et al.*, 1993). In this experiment, the difference in temperatures of leaves between the shaded and unshaded plots was relatively small (up to 3 °C) compared to the 15 °C diurnal variation in leaf temperature. Yet unshaded leaves had substantially (*c.* 45%) smaller A than shaded at midday

(Fig. 2), although Fig. 6B shows that the decrease in the morning was about 30% for a 10 °C increase in temperature. This suggests that the reduction in A was primarily related to increasing PAR flux rather than temperature *per se*. Sakai showed that midday depression of A depended on increased PAR (Sakai, 1975). It remains to be shown if the general decrease in A of all treatments was caused by the increase in PAR rather than of temperature, or if there is interaction between them.

There was substantial difference in the relationship between A and g_s between morning and afternoon. In the morning, A decreased as g_s dropped and C_i as well (Fig. 6C), but in the afternoon g_s and C_i rose but A did not (Fig. 6C). Indeed, g_s was slightly larger in the afternoon than morning (Fig. 7A) at the same temperature, so C_i would have increased (Fig. 8). The increase in g_s in the afternoon to values similar to those of the morning is not explained by the decrease in *VPD* or temperature, as both remained higher than in the morning (Fig. 1). The cause of the decreased g_s in the morning in this study is unclear. Possibly, the increase in *VPD* was responsible (Carr, 1972; Carr and Stephens, 1992), either directly or via increased transpiration, which could induce water deficits and thereby cause stomatal closure, but as no measurements were made, this possibility awaits further examination. The shade and N-treatments did not cause any differences in *VPD*, and temperature was only altered by up to *c*. 3 °C, so lack of treatment effect on g_s is explicable (and precludes an effect of PAR above a very low threshold (Fig. 7C)). More extensive experimental modification of the environment is required to separate the interacting environmental factors in the field and fully establish the relative importance of the factors in controlling g_s.

Tea has considerably larger maximum g_s, g_{smax}, (0.8 mol m^{-2} s^{-1} in the early morning, Fig. 3), but similar maximum rates of A, A_{max}, (8–11 μmol m^{-2} s^{-1}) to other woody plants (trees and shrubs g_{smax}, 0.2 mol m^{-2} s^{-1} and A_{max} 9 μmol m^{-2} s^{-1} with a range 7–12 μmol m^{-2} s^{-1}; Körner, 1995). Values from the later morning may be more comparable to measurements on other plants; g_s for tea is close to 0.2 mol m^{-2} s^{-1} at 10.00 h. The ratio A_{max}/g_{smax} (μmol mol^{-1}) is 0.041 for 55 tree and shrub species. The value for tea is 0.0125 for the largest g_s and 0.045 for the 10.00 h values. Comparable values for herbaceous species are: g_{smax} *c*. 0.3 mol m^{-2} s^{-1} and A_{max} 19 μmol CO_2 m^{-2} s^{-1} (Loreto *et al*., 1992; Wullschleger, 1993; Körner, 1995; Badeck *et al*., 1997). Thus, tea is typical of trees with low rates of A and small g_s. Internal conductance (g_i) of leaves of woody species is about 0.15 (range 0.01–0.28) mol m^{-2} s^{-1}, smaller than those of herbaceous species (mean of 0.48, range 0.3–0.7 mol m^{-2} s^{-1}). With small g_i the ratio of CO_2 in the chloroplast (C_c) to C_i is low; a value of *c*. 0.47–0.5 for C_c/C_i is established for a range of

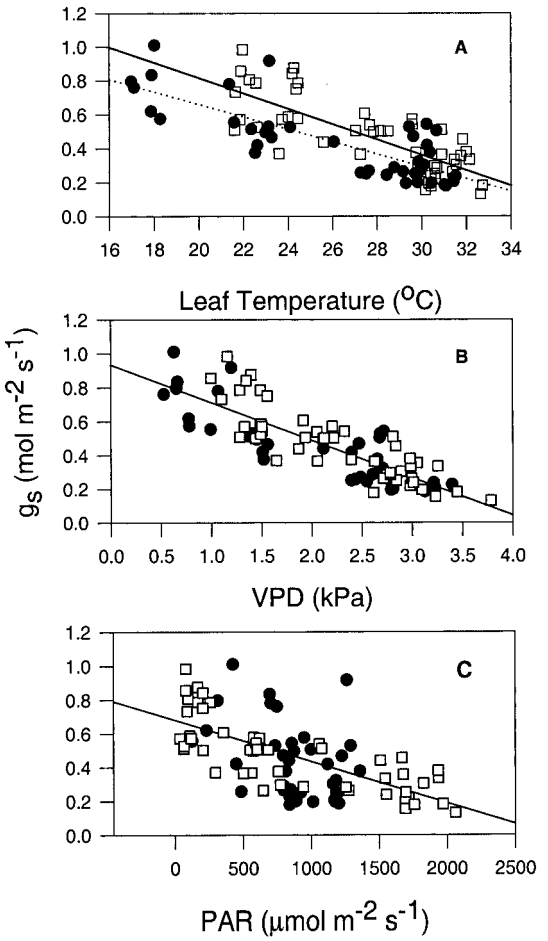

Fig. 7. Relationship between stomatal conductance (g_s) and (A) leaf temperature, (B) vapour pressure deficit (*VPD*) of the air and (C) photosynthetically active radiation (PAR), for leaves grown under different shade and N treatments in the field. All the data for different N and shades have been pooled and analysed for the morning (●) and afternoon (□) (before and after 12.00 h local time, respectively). (A) Regression of g_s on leaf temperature (morning $g_s = -0.037T_L + 1.39$: $r^2 = 0.63$, and afternoon, $g_s = -0.046x + 1.72$: $r^2 = 0.61$; regressions significantly different. (B) Regression between g_s and *VPD*, $g_s = -0.223VPD + 0.94$: $r^2 = 0.70$; regressions of relationships in the morning and afternoon not significantly different. (C) Regression of g_s on PAR, $g_s = -0.0002\text{PAR} + 0.681$: $r^2 = 0.60$; regressions for morning and afternoon not significantly different.

trees and shrubs compared to 0.59 for herbaceous species (Badeck *et al*., 1997). By analogy, tea may have low C_c, so making it susceptible to PI at large PAR flux.

The C_i is an important factor in the regulation of A. Decrease in g_s during the morning, when A was large, lowered C_i (Figs 3B, 8). Calculation of C_i is subject to a number of potential errors, particularly heterogeneous ('patchy') stomatal conductance (Weyers and Lawson, 1997). This has not been analysed in tea and further work is required to establish if there is any effect. However, it is probably of limited importance (Weyers and Lawson, 1997). Decreasing C_i during the morning would have restricted A, and as PAR rose, the excess energy caused PI. This persisted during the afternoon, even when C_i was

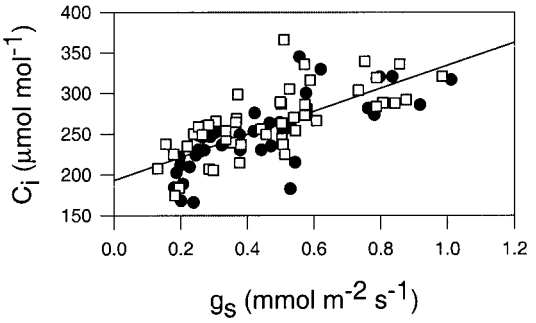

Fig. 8. Relationship between intercellular CO_2 concentration (C_i) and stomatal conductance (g_s) measured during the day on leaves of tea, grown in a field experiment with different shade and N treatments. Data for different N and shades have been pooled. The regressions for all data is $C_i = 143.70 g_s + 185.45$: $r^2 = 0.54$. Regressions for the morning (●), and afternoon (□) are not significantly different.

large so the supply of CO_2 did not limit A. The relationship between C_i and g_s (Fig. 8) was linear and identical in the morning and afternoon. Given the low A in the afternoon, it is expected that C_i would increase, at a given g_s, compared to the morning. Also stimulation of photorespiration and day-respiration by higher T_L would increase C_i, and decrease A. Hence the constant C_i/g_s is not explicable.

Photoinhibition progressed in all treatments during the morning: the q_{NP} data showing that energy dissipation rose substantially as energy accumulated in the light-harvesting system, presumably due to lack of CO_2. However, PI was exacerbated in unshaded leaves at large PAR flux, indicated by the decreased F_v/F_m ratio at approximately midday when the decrease in A was already apparent. Possibly, q_{NP} was insufficient to cope with the energy. The F_v/F_m in intense radiation was substantially smaller than in shade in the zero-N treatment, but large-N application decreased the effect. The F_v/F_m ratio was not measured progressively during exposure to intense radiation, so it is not known if the decline in A was related only to change in F_v/F_m. Photoinhibitory loss of photosynthetic capacity by inhibition of PSII has been extensively documented for plants exposed for prolonged periods to large photon flux (Baker and Bowyer, 1994). Damage to photosystem II is the probable cause of PI (Melis, 1999), as a consequence of the excessively reduced state of the primary quinone acceptor (Q_A) of electrons from PSII. This is probably the situation in tea, when electron transport to acceptors, primarily CO_2 in photosynthesis, is restricted.

The N-treatments, although substantially different, did not affect photosynthetic capacity greatly (20% decrease, not significant), and the maximum rates of A in the early morning (Fig. 2) were similar. Probably N from the soil was sufficient to maintain the N supply. However, the decrease in A of unshaded leaves during the day was greater in the low and medium N-treatment than in the high. Dissipation of energy by q_{NP} was greater in unshaded leaves than shaded in the high-N, but not in the zero-N treatment. These effects suggest that supply of N adequate for the formation of the energy-utilizing mechanisms increased the capacity of leaves to utilize energy as PAR increased. Medium and high N applications maintained q_P, even after prolonged exposure to intense radiation, although A was decreased, suggesting that mechanisms other than net CO_2 assimilation function in high light to quench excitation energy. Photorespiration is the primary route in C_3 plants, and increases with rising temperature. Synthesis of large concentrations of secondary metabolites (which are particularly important in tea and the basis of its use as a beverage), is probably insignificant as a sink for electrons and energy. This may relate to the role of N in the maintenance of A in tea, and agrees with the conclusion of Ramalho *et al.* that N-availability is a key factor in acclimation to high light in coffee (Ramalho *et al.*, 1997). They showed that a large N-supply increased photosynthetic rate and capacity and decreased PI in coffee plants grown with different N-treatments in pots. The protective effect of high N supply was attributable to increased content of xanthophyll pigments (which had a lower epoxidation state) and q_{NP}. In these experiments, the decrease in A of leaves fully exposed to light, particularly in the zero-N treatment, may be related to the accumulation of soluble carbohydrates, suggesting that assimilate production exceeded sink capacity. Carbohydrate accumulation may inhibit photosynthetic metabolism by feedback mechanisms (Paul and Driscoll, 1997), and result in the accumulation of excitation energy, thus increasing the potential for PI (Melis, 1999). However, this explanation is not satisfactory: carbohydrate accumulation would inhibit A and increase C_i. Rather, it is concluded that inadequate CO_2 availability was the main cause of PI.

In conclusion, tea grown in the field at high elevation in Sri Lanka, suffered progressive decrease in A during the morning, as radiation, T_L and *VPD* increased, which was not restored in the afternoon as they decreased. This may be explained as follows. In the early morning, with low PAR, T_L and *VPD* (compared to the afternoon), g_s was large and A low, so that C_i was high. With increasing radiation, A increased to a maximum. As temperature and *VPD* rose, g_s decreased, but relatively less than A, so C_i also decreased. During this period q_P decreased and q_{NP} increased, as the energy absorbed was not used in photochemistry. Excess energy induced PI, which persisted during the afternoon so preventing A increasing when PAR and C_i were favourable. The PI was removed during the night, and recovery was independent of N supply and shade as A was very similar in all treatments in early morning. Shade decreased the PAR flux and thus accumulation of energy, decreasing PI and maintaining A.

Abundant N increased A and decreased PI, thus stimulating assimilate production. Photoinhibition of A in tea may be decreased by shading and application of N-fertilizers.

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