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Equilibration Study to Determine Procedure for Determining Net Photosynthesis and Photon Flux Densities Response Curves

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Abstract: The wheat plants were grown in growth rooms, which were maintained at 24/16°C day and night temperatures with a photoperiod of 16 h. The gas exchange measurements were taken on fully expanded leaves of randomly selected wheat plants raised in the growth rooms. Gas exchange data were recorded using the data logger of the Infrared Gas Analyzer at 30 sec and then at 1, 2, 3, 4, 5, 7, 10, 15, 20, 25 and 30 min after enclosure. A neutral light filter was then placed on the Parkinson leaf chamber window and further readings were taken at the above time intervals. This process was repeated so that photon flux density was decreased in a step-wise manner from 1750, 1300, 1050, 600 and 275 to μmol m^{-2} s^{-1} photosynthetically active radiation. Changes in leaf temperature (T_l), net photosynthesis (Pn), stomatal conductance (g_s), transpiration rate (E) and sub-stomatal CO_2 level (Ci) were recorded following initial enclosure and subsequent changes in light intensity. T_l progressively increased over the time after initial enclosure at 1750 μmol m^{-2} s^{-1} PAR, however, the differences in T_l between times were not significant. Pn and Ci showed an initial rapid response and then a more gradual response but attained new constant values within 3-5 min of enclosure. g_s and E increased during the first 15-20 min after enclosure and then remained constant.

Key words: Equilibration, net photosynthesis, photon flux densities, response curves

INTRODUCTION

Light is a major determinant of rate of photosynthesis and fluctuates seasonally, daily and hourly during the wheat-growing season. In the previous experiment net photosynthesis (Pn) and other related parameters were determined on plants *in situ* under natural conditions when selected fully expanded (Iqbal, 1992). The observed values of Pn had a highly coefficient of variation and at least a part of this variation could be due to variation in light intensity at the time measurements. Consistent readings of Pn can be obtained in *situ* with little variation of natural light intensity anywhere as long as there is no cloud. Different workers have reported that plants exposed to full sun on cloudless days generally receive light intensity between 1700-2000 μ mol m^{-2} s^{-1} PAR (e.g. Sharma and Singh, 1989; Ziska *et al*., 1990). However, cloudless conditions can rarely be guaranteed in the countries like United Kingdom. Light conditions below saturations for wheat (c.1550 μmol m^{-2} s^{-1} PAR) are commonly in many wheat-growing regions (Blum, 1990).

When using Infrared Gas Analyzer (IRGA) to determine Pn, different workers have used different techniques. One problem is to decide how soon after enclosure Pn measurements should be recorded. Gorham, Wyn Jones and Bristol (1990) argued that measurements in bright light should be taken with in 30 sec of enclosure to avoid stomatal closure. Austin (1982) took measurements after 20-100 sec under field conditions. Yeo *et al*. (1985) enclosed rice leaves for 60-90 min before measuring Pn to allow transpiration to stabilize. Conversely, Henson *et al*. (1990) achieved steady states of CO_2 and water vapour exchange within 1 or 2 min of enclosure. Blum (1985) reported steady states of CO_2 exchange and transpiration after 5 min but preferred to use the mean of readings taken after 5 and 15 min, although his reasons for doing this are not stated. Other workers have equilibrated leaves for 10 min (Lawlor *et al*., 1989; Blum, 1990), 20 minutes (Rawson *et al*., 1983); 30 min (Robertson and Wainwright, 1987; Kemal-ur-Rahim, 1988; Blechsmidt-schneider *et al*., 1989; Sayed *et al*., 1989) and one hour (Plaut *et al*., 1990) to achieve steady states of CO_2 exchange.

In normal air closure of stomata in response to changing environment is much more rapid than opening (Kuiper and bierhuizen, 1985). Therefore, decreasing photon flux density (I) is the way used by many workers to obtain a Pn-I response curve (Kemal-ur-Rehim, 1988; Lawlor *et al*., 1989; Blum, 1990; Plaut *et al*., 1990; Iqbal, 1992). The time allowed for adjustment of CO_2 exchange rate and other parameters following decreases in light intensity has varied from 5 sec (Plaut *et al*., 1990) to between 12 and 15 min (Blum, 1990). Therefore as apart of the experiments here a preliminary investigation was conducted to determine:

1) How long it took leaf temperature (T_1), Pn, stomatal conductance (g_s), Transpiration rate (Ci) to reach steady state values after a leaf was placed in the light chamber,
2) And how long it took these variables to respond to subsequent decreases in I.

MATERIALS AND METHODS

The gas exchange measurements were taken on fully expanded leaves of randomly selected wheat plants raised in the growth rooms.

Walk-in growth rooms: The wheat plants were grown in two growth rooms which were maintained at 24/16°C day and night temperatures with a photoperiod of 16 hours. A bank of 125W universal warm white fluorescent tubes provided light. There were 24 tubes in each growth room and they provided approximately 200-300 μmol m^{-2} s^{-1} PAR at the leaf surface. The relative humidity in the growth rooms ranged 40-65 percent.

Light chamber: A light chamber was constructed using aluminum framework blocked in by polystyrene sheets. These were covered in aluminum foil on the inside to maximize light reflection. Access to the chamber was through an open front, which was screened by a sheet of heavy gauge black plastic to protect the experimenter. In order to provide a constant high light intensity source (Above 2000 μmol m^{-2} s^{-1} PAR) for gas exchange (GER) measurements; a 1000 W mercury fluorescent lamp (GEC) was used. This was suspended from the ceiling above the chamber by a chain and shielded in an aluminum cylinder to focus light inside the chamber. The incoming light was passed through a water fed heat filter to prevent excessive increase in temperature in the Parkinson leaf chamber (PLC). The PLC was fixed on movable retort stand in a horizontal position in the light chamber below the heat filter and at right angle to the light source. The air inlet and outlet tubes ran from the PLC to the IRGA and volumetric air pump, which were outside the light chamber. Reference air was drawn down a tube attached at a height of 15 m to a pole outside the building. This equipment was installed inside a walk-in growth room, separate to the one where the experimental plants were growing.

The growth room lights, air conditioning system and 1000 W lamp were switched on for half an hour before readings commenced to allow the lamp to come-up to its desired PAR level and growth room to its operating temperature (25 ± 2°C). The fan in the PLC and air supply pump was also switched on the purge the humidity from the system.

Collection of gas exchange data following initial enclosure and subsequent decreases in photon flux density: The plants plus polystyrene lid were lifted off their original containers in the walk-in growth rooms and placed on a similar empty container for transfer to the growth room containing the IRGA. Plants, supported by their polystyrene lid, were transferred to a container, which was filled with a similar concentration of nutrient solution and appropriate salinity level in the light chamber. Each time a new tray was placed inside the light chamber and leaf 3 on the main stem of a randomly selected plant was immediately placed inside the PLC. GER data were recorded using the data logger of the IRGA at 30 sec and then at 1, 2, 3, 4, 5, 7, 10, 15, 20, 25 and 30 min after enclosure. A neutral light filter was then placed on the PLC window and further readings were taken at the above time intervals. This process was repeated so that I was decreased in a step-wise manner from 1750, 1300, 1050, 600 and 275 to μmol m^{-2} s^{-1} PAR. The plants were then removed from the light chamber and returned to their original growth room position. A new set of plants was ten transferred. Eighteen readings, 6 of each of 3 salinity levels (0, 100 and 200 mol m^{-3} NaCl) were recorded in this way. To determine the effects of salinity and the time, data were analyzed using analysis of variance technique.

RESULTS

The data for the salt stressed treatments showed similar trends over time as data for the control and are therefore not presented. By running the pump with the PLC closed and measuring relative humidity (RH) it was found that it took 10-30 sec for the pump to purge the chamber and tubing. Therefore, no accurate readings could be obtained until at least 30 sec had elapsed. This problem did not reoccur when the Pn was measured following subsequent decreases in light intensity as the leaf was left undisturbed in the PLC.

Changes in T_1, Pn, g_s, E and Ci at 0 mol m^{-3} NaCl following initial enclosure and subsequent changes in light intensity are represented in Fig. 2-5. In these figures each data point is the mean of six leaves. T_1 progressively increased over the time after initial enclosure at 1750 μmol m^{-2} s^{-1} PAR, however, the differences in T_1 between times were not significant (Fig. 2a). Pn (Fig. 2b), g_s (Fig. 2c) and E (Fig. 3a) increased during the first 15-20 minutes after enclosure and then remained constant. Conversely Ci decreased during the first 15-20 min and thereafter remained constant (Fig. 3b). Differences between times were significant for all the parameters.

When photon flux density was stepped down (for example from 1050 to 600 μmol m^{-2} s^{-1} PAR), T_1 decreased

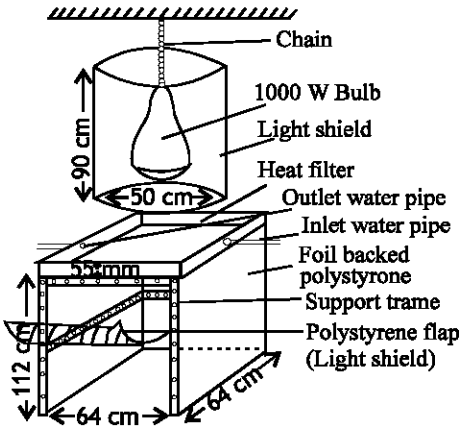

Fig. 1: Light chamber, showing position of light source, heat filter and support frame

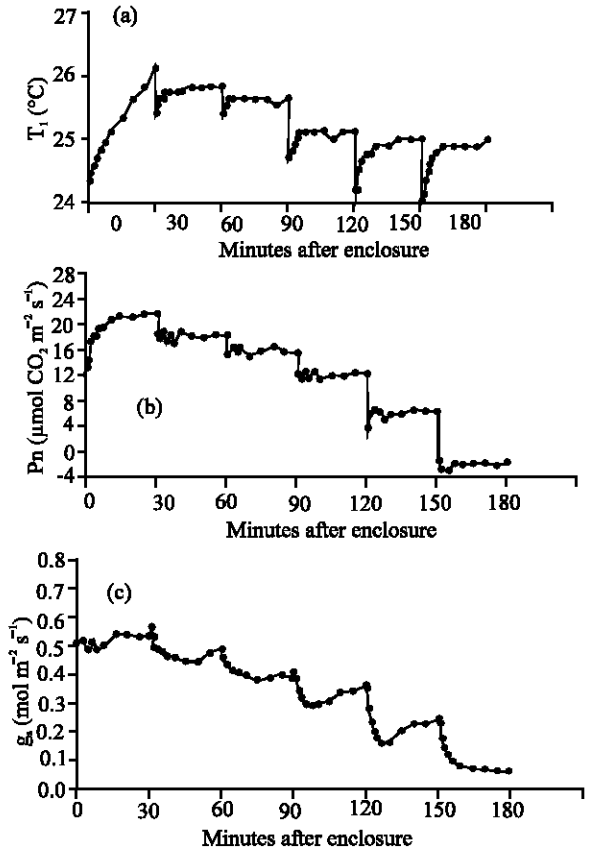

Fig. 2: Change in (a) leaf temperature (T_1), (b) net photosynthesis (Pn), (c) stomatal conductance (g_s) following enclosure in the leaf chamber and step-wise decreases in light intensity form 1750, 1300, 600 and 275 to 0 μmol m^{-2} s^{-1} photosynthetically active radiation at 30 min intervals

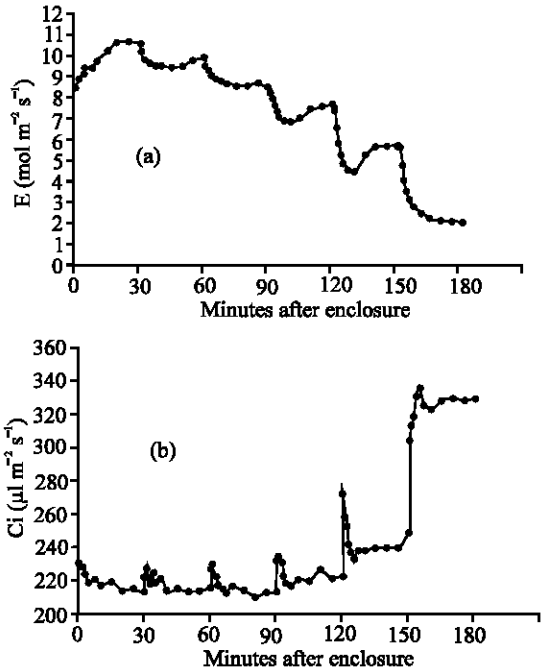

Fig. 3: Changes in (a) transpiration rate (E), (b) sub-stomatal CO_2 concentration (Ci) following enclosure in the leaf chamber and step-wise decrease in light intensity from 1750, 1300, 600 and 275 to 0 μmol m^{-2} s^{-1} photosynthetically active radiation at 30 min intervals

rapidly and there after it increased and reached a new constant value within 15-20 min (Fig. 4a). Pn adjusted rapidly within 1-2 min (Fig 4b). For g_s and E, an initial rapid decrease was observed followed by a slower increase (Fig. 4c and 5a, respectively), suggesting that 30 min was not long enough for these parameters to reach new constant values. Pn and Ci followed a similar pattern as at highest light intensity (Fig. 4b and 5b, respectively). Similar trends were noted when I was stepped down from 275 to μmol m^{-2} s^{-1} PAR.

DISCUSSION

The study showed that T_1 increased progressively over time after enclosure. The increase in T_1 was mainly due to high I and consequently higher temperature in the light chamber than in growth room where the plants were growing. The light power lamp had a heating effect despite the presences of the heat shield running cold water in between the light and the PLC. However, the increase in T_1 over time was not significant. Following enclosure Pn and Ci showed an initial rapid response and then a more gradual response but attained new constant

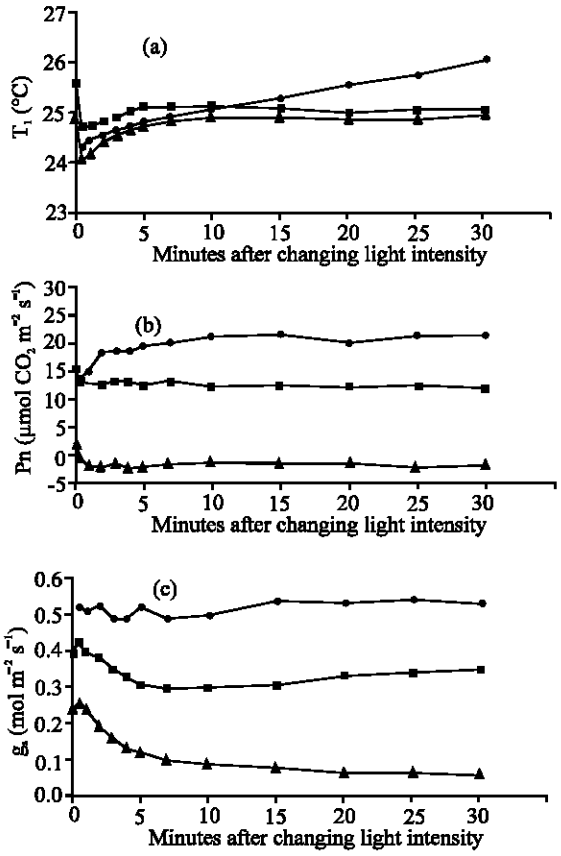

Fig. 4: Values of (a) leaf temperature (T_l), (b) net photosypthesis (Pn) and (c) stomatal conductance (g_s) at 1750 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) following placement in the light chamber (●), subsequent decreases in light intensity from 1050 to 600 (■) and 275 to 0 (▲) μmol m^{-2} s^{-1} PAR

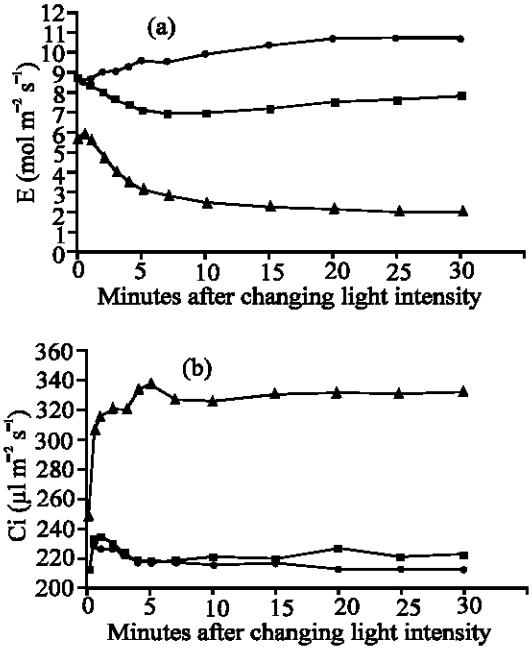

Fig. 5: Values of (a) transpiration rate (E), (b) sub-stomatal CO_2 concentration (Ci) at 1750 μmol m^{-2} s^{-1} photosynthetically active radiation (PAR) following placement in the light chamber (●) and subsequent decrease in light intensity from 1050 to 600 (■) and 275 to 0 (▲) μmol m^{-2} s^{-1} PAR

values within 3-5 min of enclosure. Similar results were reported by Kemal-ur-rahim (1988). g_s and E took longer than 15 min to reach new constant values after enclosure suggesting that stomata took time to adjust from low I in the growth room where plants were growing to very high I in the light chamber. These results indicated that Pn, g_s, E and Ci need at least 15-20 min "equilibration time" (i.e. the time taken to reach constant values after initial placement in the light chamber). It was therefore, decided that the most appropriate method of equilibration to use would be to leave plants in the light chamber for approximately 20 min before commencing measurements. This procedure is similar to that used by other workers (Rawson *et al*., 1983); Robertson and Wainwright, 1987; Kemal-ur-rahim, 1988; Blechschmidt-Schneider *et al*., 1989; Sayed *et al*., 1989; Iqbal, 1992) who also used 20-30 min equilibration time.

After decreasing I, Pn in the test leaf adjusted within 1-2 min. Similar results were also reported by Kemal-ur-Rahim (1988). Plaut *et al*. (1990) use a 'response time' (i.e. the time taken to reach new constant values following a decrease in I) of seconds, which on the basis of the results obtained here is not long enough for Pn to adjust to the new I. Blum (1990) used a response time of 15 min. Use of such a long response time would limit the number of leaves that could be measured in a day and on the basis of the results obtained here, would also be long enough for g_s and E to reach new constant values.

The slower response of g_s, E and Ci after changing I suggests that subsequent measurements at lower light intensities would require at least 30 minutes response time in order to be able to develop g_s-I, E-I and Ci-I response curves. However, in future experiments, a response time of 3 min was used. This long period was considered to be long enough for Pn to adjust to the new light intensity, but not for g_s, E and Ci. During this study no difference in equilibration and response times between treatments was observed (data not presented). Therefore, the same procedure for all treatments was adapted for Pn-I response curves.

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