

Diurnal patterns of chlorophyll fluorescence and CO₂ fixation in orchard grown *Torreya taxifolia* (Arn.)^{1,2}

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KOEHN, A.C. (Alabama A&M University, Normal, AL 35762) AND R.L. DOUDRICK (Southern Institute of Forest Genetics, Saucier, MS 39574). Diurnal patterns of chlorophyll fluorescence and CO₂ fixation in orchard grown *Torreya taxifolia* (Am.). Bull. Torrey Bot. Soc. 126:93-98, 1999.—Chlorophyll fluorescence and CO₂ fixation measurements were taken on sunny days **in October, 1996, on three *Torreya taxifolia* (Am.) plants grown in an open canopy orchard.** Information from chlorophyll fluorescence quenching analysis indicated during periods of highest light intensity and temperatures there were reductions in yield of photosystem II photochemistry and photochemical quenching and increases in nonphotochemical quenching. Photochemical quenching and yield of photosystem II recovered by the end of the measurement period in late afternoon to levels measured at the beginning of the day. Nonphotochemical quenching remained at high levels for a longer period of time and by late afternoon had not returned to levels measured at the beginning of the day. Diurnal patterns of CO₂ fixation and stomatal conductance showed decreases in the afternoon as ambient temperatures remained high and light intensity began to decrease. Internal CO₂, partial pressures remained constant throughout most of the day, possibly indicating the presence of nonstomatal limitations to photosynthesis. The measurements of CO₂ fixation and chlorophyll fluorescence on the three *T. taxifolia* plants in this study indicate that the plants recovered from daily periods of high light and temperatures suggesting that they may tolerate higher light conditions than found in their native habitat.

Key words: CO₂ fixation, photochemical quenching, nonphotochemical quenching, yield of photosystem II, *Torreya taxifolia*.

Torreya taxifolia (Am.), Florida torreya, was **once a subcanopy small tree common along the Appalachian River in the central panhandle of Florida (Schwartz and Hermann 1993). It suffered a catastrophic decline during the 1950's and fewer than 1500 nonreproducing plants presently grow on ravine slopes along the east-em side of the river.** Schwartz et al. (1995) evaluated several theories that might account for the decline of *T. taxifolia*, including fungal pathogens, water stress, climate warming, hydrologic change, and fire suppression. They concluded that there was little evidence to suggest that the decline of the *T. taxifolia* population was a direct result of unusual environmental stress, pathogens, or fire management practices (Schwartz et al. 1995). The plants are found only in dense

shade where the light intensity is typically below the measured light saturation point, which is less than 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for *T. taxifolia* (Schwartz and Hermann 1993; Schwartz et al. 1995). Specimens, however, appear to grow vigorously in arboreta, residential gardens, and as potted plants where light intensities are much higher than in their native habitat (Workshop on the Florida torreya, Tall Timbers Research Station, Tallahassee, FL, November, 1996).

Studies on diurnal patterns of photosynthesis regulation provide insights into a plant's ability to adjust to changes in light and temperature on a daily basis and may provide clues into a plant's adaptability to its growing environment (Geiger and Servaites 1994). Our research measured chlorophyll fluorescence using a modulated fluorometer and CO₂ fixation using an open-flow photosynthesis system. These two systems provided a nondestructive means of assessing the plant's response to changes in daily temperature and light conditions. Analysis of photochemical and nonphotochemical quenching from chlorophyll fluorescence measurements were used to decide if the daily changes in light and temperature caused any physiological stress to *T. taxifolia* plants grown in an orchard environment (Krause and Weis 1991; Schreiber et al. 1994).

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This information would be useful when considering canopy environments for future reestablishment of *T. taxifolia*.

Material and Methods. PLANT MATERIAL.

Torreya taxifolia cuttings were taken in 1989 from trees in the native range, rooted (Nicholson 1993), and planted in 1992 in the hedge orchard at the Southern Institute of Forest Genetics (SIFG), Harrison Experimental Forest, Saucier, MS (89°03'W, 30°37'N, R11 T5W SE¼ of NE¼ of Section 11). Plants were protected from direct sunlight using 50% shade cloth until July, 1996, when the shade cloth was repositioned so that plants received full exposure in the morning and shading in the afternoon. The PAR (photosynthetically active radiation, 400-700nm) for full sun in October was approximately 1900 $\mu\text{mol m}^{-2}\text{s}^{-1}$. Plants were fertilized in August, 1996 using a controlled release fertilizer (Osmocote® 14-14-14, 16 gr/plant). Plants were irrigated when soil tensiometer readings reached 30-35 cbars. The soil type in the orchard is a fine, sandy loam (Soil Survey of Harrison County, Mississippi, 1975). In October, 1996, daily maximum and minimum temperatures ranged from 19°C - 31°C and 4°C - 22°C, respectively.

EXPERIMENTAL PLAN. Three *T. taxifolia* plants of similar size and vigor were selected for CO₂ fixation and chlorophyll fluorescence measurements on clear, sunny days on October 11, 15, and 19, 1996. Current year needles, attached to the plant, were placed in the LI-COR 6400 photosynthesis measurement chamber in the morning and remained in chamber throughout the day. In other words, continuous measurements of CO₂ fixation and chlorophyll fluorescence were taken on needles from plant 1067-89-F, #72 on October 11, plant 1052-89-F #42 on October 15, and plant 1067-89-F, #31 on October 19. An adapter chamber was used so the chlorophyll fluorescence probe from the PAM-2000 fluorometer could be inserted into the chamber; as a result, CO₂ fixation and chlorophyll fluorescence measurements were taken almost simultaneously on the same needles at near ambient conditions.

CO₂ FIXATION. Photosynthesis was measured using the LI-COR 6400 Photosynthesis System (LI-COR, Inc., Lincoln, NE) fitted with the 6400-06 PAM adapter chamber top. The mole fraction of CO₂ entering the leaf chamber was maintained at 370 $\mu\text{mol CO}_2 \text{ mol}^{-1}$ air using the 6400-01 CO₂ injector system (LI-COR, Inc.,

Lincoln, NE). The LI-COR 6400 was programmed so that chamber temperature followed ambient air temperature measured using a Vaisala Humitter 50Y relative humidity and temperature probe (Vaisala, Wobum, MA.) connected to the LI-COR 6400. A gallium arsenide phosphide sensor mounted near the leaf surface measured the PAR inside the chamber. The position of the chamber was adjusted throughout the day to avoid shading of the needles by the chamber walls. The flow rate of air through the system was set at 300 $\mu\text{mol s}^{-1}$ for all measurements and humidity was regulated by adjusting the air flow through the desiccant tube. The humidity thus regulated was similar to ambient relative humidity except in early morning hours when ambient humidity was high enough to cause condensation on the infrared gas analyzer, during these periods the chamber humidity was regulated to be lower than ambient. The LI-COR 6400 was programmed to record readings and match analyzers every 12 minutes; each data point on the graphs (Figs. 1-4) represents one measurement. Vapor pressure deficit of the leaf, the difference between saturation vapor pressure at leaf temperature and the vapor pressure of air, stomatal conductance, g_s , and internal CO₂ concentration, C_i , were calculated using the standard equations provided by the LI-COR 6400 software. Total needle area was calculated from photographs using the SigmaScan video analysis system (Jandel Corporation, San Rafael, CA).

CHLOROPHYLL FLUORESCENCE. Chlorophyll fluorescence was measured using a PAM-2000 Chlorophyll Fluorometer (Heinz Walz GmbH, Effeltrich, Germany). The fiberoptic probe was inserted into the top of the chamber head of the LI-COR 6400 photosynthesis system at a 60° angle to the leaf surface and the distance adjusted so the probe did not shade any of the leaf surface. Settings on the fluorometer were adjusted so that the proper saturation kinetics were achieved. Settings were considered adequate if a plateau was reached before the termination of the saturating pulse, as determined by the PAM-2000. Needles were dark-adapted for 15 minutes to determine F_v/F_m ($F_v = F_m - F_o$) before other measurements were taken in the morning. Yield, photochemical quenching, and nonphotochemical quenching were recorded at 12 minute intervals by the PAM-2000 immediately after the LI-COR 6400 logged CO₂ fixation information. F_v' was measured during illumination by far-red light immediately following the saturating light

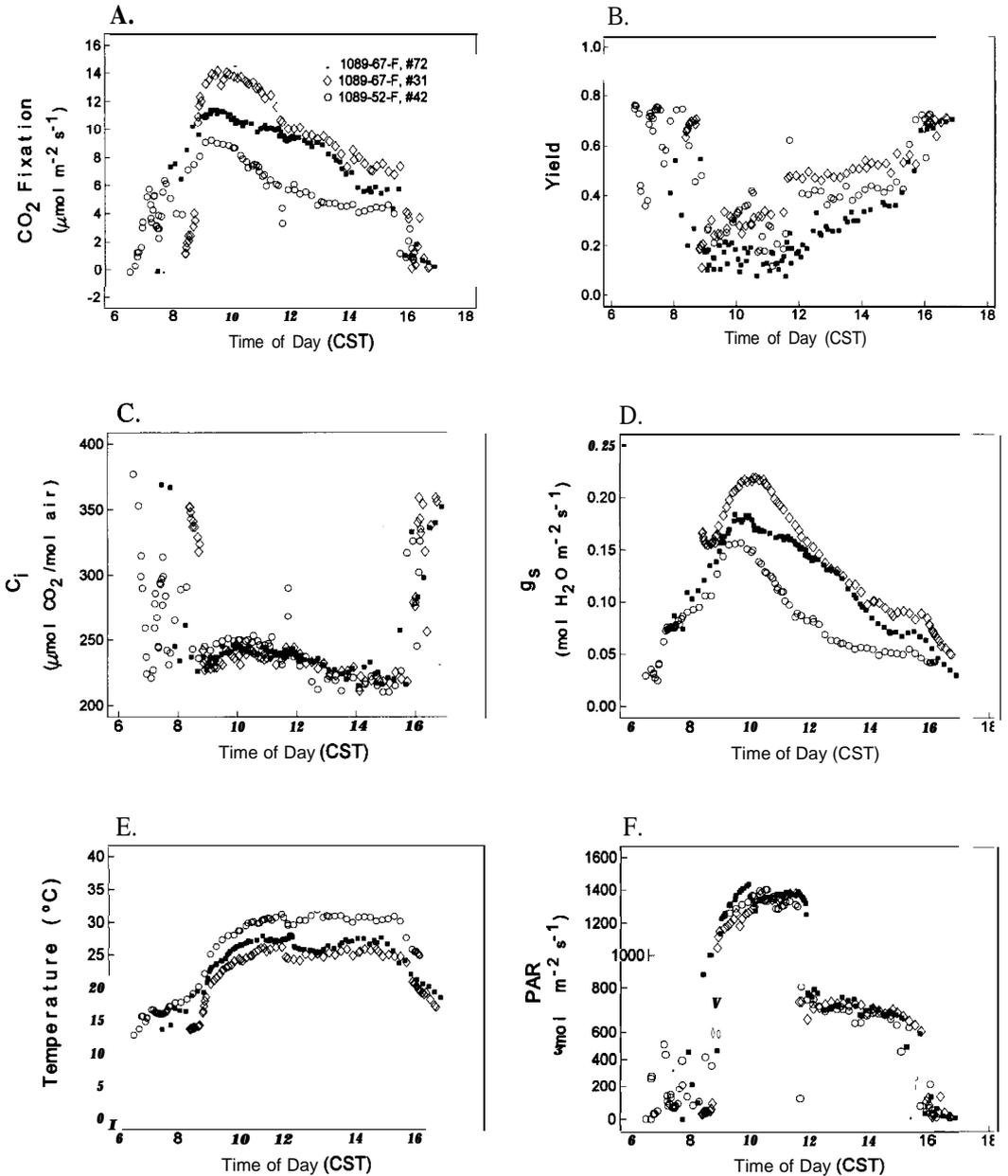


Fig. 1. Diurnal patterns for three *Torreya taxifolia* plants measured in October, 1996. CO₂ fixation, C_i, and g_s were calculated using the LI-COR 6400 photosynthesis system calculations. Temperature ($^{\circ}\text{C}$) represents the temperature measured near the leaf surface, and PAR (photosynthetically active radiation) was measured inside the leaf chamber. Yield represents the apparent efficiency of PS II photochemistry calculated from chlorophyll fluorescence measurements. Symbols for all graphs are listed in Fig. 1A.

pulse and was used for calculating photochemical quenching and nonphotochemical quenching. Yield, photochemical quenching, and nonphotochemical quenching were calculated according to Genty et al. (1989) and Schreiber et al. (1986). Yield is defined as the apparent ef-

iciency of photosystem II (PS II). Photochemical quenching, qP, is attributed to increased rates of Q_A oxidation, Q_A is a quinone-type electron acceptor in the PS II reaction center; as Q_A becomes reduced, qP decreases. Nonphotochemical fluorescence quenching, qN, reflects the frac-

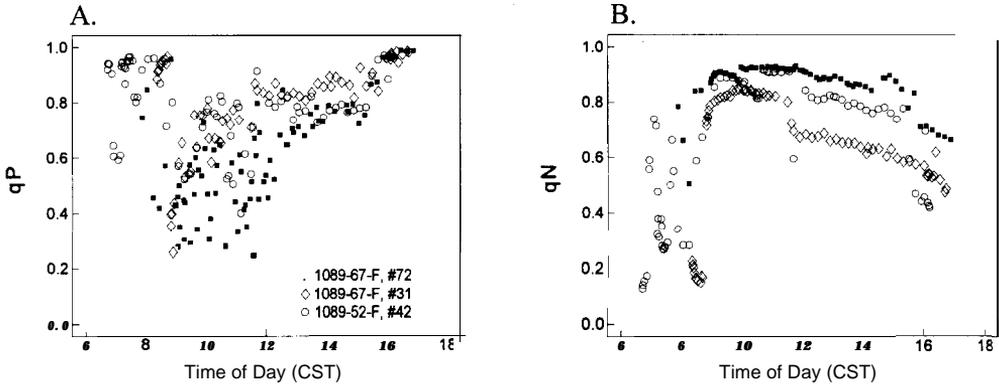


Fig. 2. Photochemical (qP) and nonphotochemical (qN) quenching for three *Torreyataxifolia* measured in October, 1996. As qP decreases, Q_A , the quinone-type electron acceptor in PS II reaction centers, becomes reduced. Increases in qN indicate an increase in radiationless energy dissipation, usually in the form of heat. Values for qP and qN may range from 0.0 to 1.0. Symbols represent the plants as listed in the legend of Fig. 2A.

tion of light energy primarily released as heat and not used in photochemistry (Genty et al. 1989; Krause and Weis 1991; Walker 1992; Horton et al. 1994; Valladares and Pearcy 1997). Increased qN may be involved in reducing rates of photosynthesis and can affect a plant's ability to respond to rapidly changing daily temperatures (Valladares and Pearcy 1997).

Results and Discussion. CO₂ fixation in *T. taxifolia* plants measured in October, 1996, peaked at about 1000 (CST) when PAR was approximately 1250-1400 $\mu\text{mol m}^{-2}\text{s}^{-1}$ and then declined even though PAR remained high until 1100 (Figs. 1A, 1F). Yield and qP decreased as CO₂ fixation increased (Figs. 1B, 2A). During periods of high PAR, greater than 1200 $\mu\text{mol m}^{-2}\text{s}^{-1}$, qP dropped below 0.6 (Fig. 2A). Results

from a study by Öquist et al. (1992) on different light acclimated species indicated that plants may become photoinhibited when qP drops below 0.6. If so, *T. taxifolia* plants in our research would have experienced some photoinhibition from about 0900 until 1200. During this time, i.e. 0900-1200, qN was high, about 0.9 (Fig. 2B), when temperatures (Fig. 1E), PAR (Fig. 1F), and vapor pressure deficits (Fig. 3) were high, qN then declined slowly in the afternoon. By dissipating energy during periods of reduced CO₂ fixation, qN may serve as a protective mechanism that prevents damage to the PS II reaction centers (Demmig and Winter 1988; Genty et al. 1989; Ögren 1991; Demmig-Adams and Adams 1992; Ruban and Horton 1995).

Figures 2B and 4 show hysteresis for two plants in the diurnal pattern of qN; i.e., it was higher in late afternoon, approximately 0.4 to 0.6, than measured in the morning, approximately 0.1, despite a drop in irradiance levels (Fig. 1F). Chlorophyll fluorescence measurements were started later in the morning for the third plant and PAR values were already high by the time we started recording data; therefore, there was no demonstration of hysteresis in its data even though qN was sustained in the afternoon. Hysteresis may become more accentuated in qN as the length of time at high irradiances increases (Demmig and Winter 1988). Adams et al. (1989) suggest that because of reduced photosynthesis rates, plants are subjected to excessive light energy and therefore, more susceptible to sustained changes in qN. If qN is a protective mechanism, it would seem logical that it should

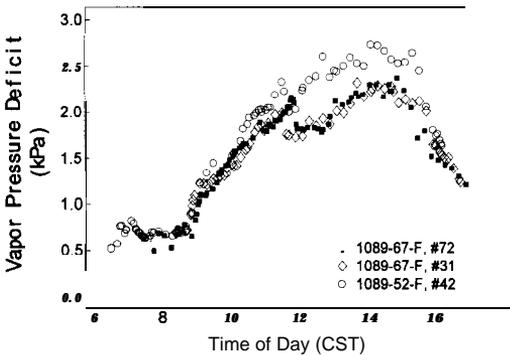


Fig. 3. Diurnal pattern for vapor pressure deficit calculated for each *Torreyataxifolia* plant measured in October, 1996. The symbols are the same as for Figs. 1 and 2.

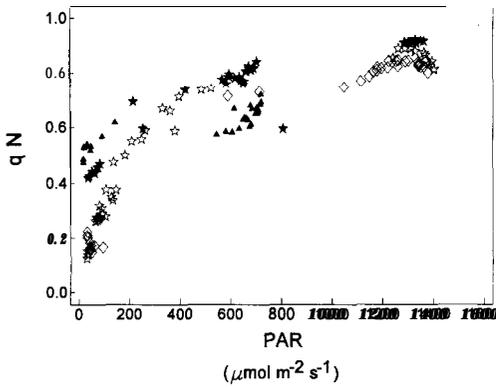


Fig. 4. Diurnal data from Fig. 2B versus PAR from Fig. 1F for two *Torreya taxifolia* plants demonstrates hysteresis in qN . Symbols are as follows: 0 = increasing light intensity measurements for 1089-67-F #31, A = decreasing light intensity measurements for 1089-67-F #31, \star = increasing light intensity measurements for 1089-52-F #42, \blackstar = decreasing light intensity measurements for 1089-52-F #42.

remain sustained until light, temperature, and vapor pressure deficit extremes are reduced, thereby increasing its effectiveness as a protective mechanism for PS II reaction centers. In two of the three plants measured, there was a rapid rate of decline in CO_2 fixation and drop in qN with the sudden reduction in PAR in the afternoon (Figs. 1A, 1F, 2B). The explanation for this is that chlorophyll fluorescence quenching is closely associated with light environment and the regulation of photosynthesis (Horton et al. 1994; Krause and Weis 1991). CO_2 fixation rates and qN for the third plant responded less abruptly with the sudden decrease in PAR in the afternoon; we are unable at this time to explain the different response of the third plant to the reduction in PAR.

If CO_2 fixation rates remain high when stomates close, then C_i decreases. If, however, CO_2 fixation is reduced by nonstomatal factors, then the demand for CO_2 decreases, stomates close, and C_i within the leaf will remain the same (Wise et al. 1991, Kozłowski and Pallardy 1997). Another explanation for constant C_i with declining CO_2 fixation rates may be patchy stomatal closure (Wise et al. 1992). Stuhlfauth et al. (1988) attributed constant rates of C_i in *Digitalis lanata* EHRH to nonstomatal factors when CO_2 fixation was reduced in parallel with g_s . A similar situation occurred in the three *T. taxifolia* plants measured in this study, CO_2 fixation and g_s decreased while C_i remained almost constant from 0900 until 1500 hrs. (Figs 1A, 1C, 1D)

indicating the possibility of the presence of either nonstomatal factors that may reduce CO_2 fixation or patchy stomatal closure.

The photochemical system of the three *T. taxifolia* measured in this study effectively regulated energy absorption and dissipation during periods of high PAR, vapor pressure deficits, and temperature. Although qN changed slower than other factors measured, it was near zero at the beginning of daily data collection. Values of qP dropped below 0.6 for periods of time, but recovered by the end of the measurement period. The ratio of F_v/F_m that is used to detect photoinhibition in plants was in the normal range for unstressed plants, 0.77-0.83 for the three plants when measured early in the morning (Björkman and Demmig 1987). The value of 0.77 is low; however, CO_2 fixation data indicate there was no photoinhibition.

This study has measured physiological responses to diurnal variations in light, temperature, and vapor pressure deficit in three *T. taxifolia* plants. The plants appeared to have acclimated to the change in light conditions that occurred when the shade cloth was repositioned in July, 1996. Future research should include establishing *T. taxifolia* plantings using different canopy structures. Variables to consider when defining canopy structure would be the intensity of direct and indirect sunlight reaching the level of the canopy containing the *T. taxifolia* plants and the time during the day when the plants receive direct and indirect sunlight. Measurements of quenching analysis from chlorophyll fluorescence measurements combined with CO_2 fixation data should allow us to assess the adaptability of *T. taxifolia* to interactions of light, vapor pressure deficit, and temperature in these various environments. This type of information should help in solving the problem of successful reintroduction of *T. taxifolia* in its native range since there are questions about the environmental conditions necessary for vigorous growth and reproduction of *T. taxifolia* plants. As is the case for all organisms, the ability of *T. taxifolia* to survive environmental stresses, compete effectively with other organisms, and reproduce, will determine its success in avoiding extinction (Levontin 1978).

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