An Ecophysiological Analysis of Shade Effects on *Clematis hirsutissima* var. *arizonica*

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Abstract: Net photosynthetic rates and water potentials of adult *Clematis hirsutissima* var. *arizonica* were compared among four canopy density environments (open, medium, closed, experimentally closed) in a *Pinus ponderosa*-dominated forest in northern Arizona on four dates in summer 1994. Daytime water potential was lower (greater water stress) in open versus shaded environments, with little difference in pre-dawn water potential among environments. Net photosynthetic rate was higher in open versus shaded environments on most dates. The results indicate that shading ameliorated daytime water stress and that net photosynthetic rate was more strongly limited by low light intensity in shaded environments than daytime water stress in open environments. Regulation of *Clematis* physiology by canopy density is discussed in relation to habitat requirements for regeneration and population growth.

Introduction

Clematis hirsutissima var. *arizonica* (Heller) Erickson (referred to as *Clematis* hereafter) is considered a sensitive species by the U.S. Forest Service and is a candidate for federal protection under the U.S. Endangered Species Act. The species is an herbaceous perennial shrub with pinnately compound leaves, fine leaflets, and solitary, purple, bell-shaped flowers (Kearney and Peebles 1960). The stem dies back in the fall after seeds are shed. Populations of *Clematis* grow at high elevations (2300–2800 m) near Flagstaff, Arizona, the Chuska Mountains of Arizona, and the Zuni Mountains of New Mexico. The known populations have a patchy distribution and are geographically rare.

One of the major anthropogenic factors hypothesized to have limited the distribution of *Clematis* in northern Arizona is the removal of overstory shade by timber harvesting. Concerns over the detrimental effects of harvesting overstory *Pinus ponderosa* Lawson on *Clematis* are substantiated by recent surveys indicating that approximately 85 percent of all *Clematis* populations in northern Arizona occur in habitats where average light levels are below 50 percent full sun (Maschinski 1989), and by measurements of lower bud mortality, greater seed set, greater production of viable seed, and greater rates of seedling establishment in shaded versus open habitats (Maschinski et al. in press). These data suggest facilitation of *Clematis* reproduction by shading from the *Pinus ponderosa* canopy. Because *Clematis* grows in a geographic region characterized by low water availability, high evaporative demand, and high light intensity, shade may be beneficial to *Clematis* reproduction and seedling survival by moderating water stress, as has been documented for understory or herbaceous species in other regions (e.g., Knapp et al. 1989, Knapp and Smith 1990, Schultz and Adams 1995). In addition, shade may stimulate *Clematis* reproduction and survival by reducing limitations on net photosynthesis by water stress. In this paper, we test the hypothesis that shading ameliorates water stress of *Clematis* in northern Arizona. We also examine the role of water stress and light intensity in regulating net photosynthesis of adult *Clematis* under field conditions.

Methods

In 1991, we located and surveyed *Clematis* populations near Lower Lake Mary approximately 25 km southeast of Flagstaff, Arizona. All populations occur in a forest dominated by *Pinus ponderosa*. We categorized these populations into three environments based on the average percentage of full sun penetrating to the forest floor (see Maschinski et al., in press, for additional details): (1) open environments with light levels greater than 75 percent ("open"), (2) medium environments with light levels between 50 and 65 percent ("medium"), and (3) closed environments with light levels less than 40 percent ("closed"). In 1992, we created a fourth environment ("experimentally closed") by covering half of the open environments with wooden lattice structures to reduce light penetration to the forest floor. The lattice reduced average light intensity from 76 percent to 5 percent.

In 1994, we measured net photosynthetic rates and water potentials on fully expanded foliage of reproductively mature plants in one population of each of the four environments on the following dates: May 26, June 24, July 11, and August 8. The populations used for these measurements were representative samples of the four environments at the Lake Mary Site. Weather conditions varied among the four measurement dates. In May, the sky was overcast. In June and July, the sky was free of clouds. In August, intermittent clouds occurred.

For May, June, and July, we measured water potentials on one leaf from each of five representative plants per environment using a pressure bomb (PMS, Corvallis, OR) at predawn (0600 hours), mid-morning (AM = 0800–1000 hours), and early afternoon (PM = 1300–1500 hours) time periods. We measured net photosynthetic rate on the same plants during the AM and PM periods using a 0.25 liter cuvette and portable, closed-loop photosynthesis system (LI-6200, Li-cor, Lincoln, NE). All measurements of photosynthesis were conducted over 30 seconds under near-ambient conditions (± 10%) of temperature, light intensity, and relative humidity. We measured the area of each leaf used for photosynthesis measurements in the laboratory using an Ag-Vision imaging system (Decagon Devices, Pullman, WA). We then measured the weight of each leaf following drying for 24 hours at 60°C. The measurement procedure in August was identical to that for other dates except that persistent afternoon thundershowers prevented any PM measurements. During the photosynthesis measurements, we also measured the following environmental parameters: photosynthetic photon flux density (PPFD) assessed 0.5 m above the target plant with an 0.8 m-long PAR Ceptometer (Decagon Devices, Pullman WA); leaf and cuvette temperatures assessed with fine wire thermocouples; and cuvette relative humidity assessed with a Vasiala humidity sensor (Li-cor, Lincoln, NE). Leaf-to-air vapor pressure deficit (VPD) was calculated based on leaf and cuvette temperature and cuvette relative humidity.

Physiological and environmental data from each date were analyzed by ANOVA using SAS-Statistics software (Cary, NC) with canopy environment (open, medium, closed, experimentally closed), measurement time (AM, PM, pre-dawn for water potential), and their interaction as factors. Mean comparisons were made using the Student–Newman–Keuls test at the 0.05 level.

Results

Light levels were highest for the June and July measurements (clear sky), intermediate for August (intermittent clouds), and lowest in May (cloudy conditions) (Figure 1). Environment x time was a significant ($p < 0.05$) source of variation in June and July, reflecting inconsistent differences in light intensity between AM and PM among environments. Light intensity differed significantly ($p < 0.0001$) among canopy environments at all dates with the highest intensities occurring in the open environment and the lowest intensities occurring in the closed or experimentally closed environments (Figure 1). Light intensity did not differ significantly between closed and experimentally closed environments on any date.

Vapor pressure deficit (VPD) was greatest in June and July, intermediate in August, and lowest in May (Figure 2). Vapor pressure deficit differed significantly ($p < 0.005$) among environments at all dates and was greater in open compared with other environments. The medium canopy environment had significantly ($p < 0.05$) greater VPD than the closed and experimentally closed environments in July and August, but not on other dates. Vapor pressure deficit was generally similar for closed and experimentally closed environments. Differences in VPD among environments primarily resulted from differences in leaf temperature, which often differed by 7–10°C between open and shaded environments, rather than differences in relative humidity, which differed by only 1–3 percent. Measurement time was a significant ($p < 0.0001$) source of variation in VPD for May, June, and July, resulting from consistently greater VPD for the PM versus the AM period. Environment x time was a significant ($p < 0.02$) source of variation in June and July due to differences in VPD response over measurement times among environments.

Pre-dawn water potential in all environments decreased from May to July, suggesting depletion of soil water in the rooting zone from early to mid summer (Figure 3). Canopy environment, measurement time, and environment x time were significant ($p < 0.02$) sources of variation in water potential for all dates. Average water potential was greatest at pre-dawn, intermediate in the AM, and lowest in the PM for all dates. Although statistically significant, variation in May water

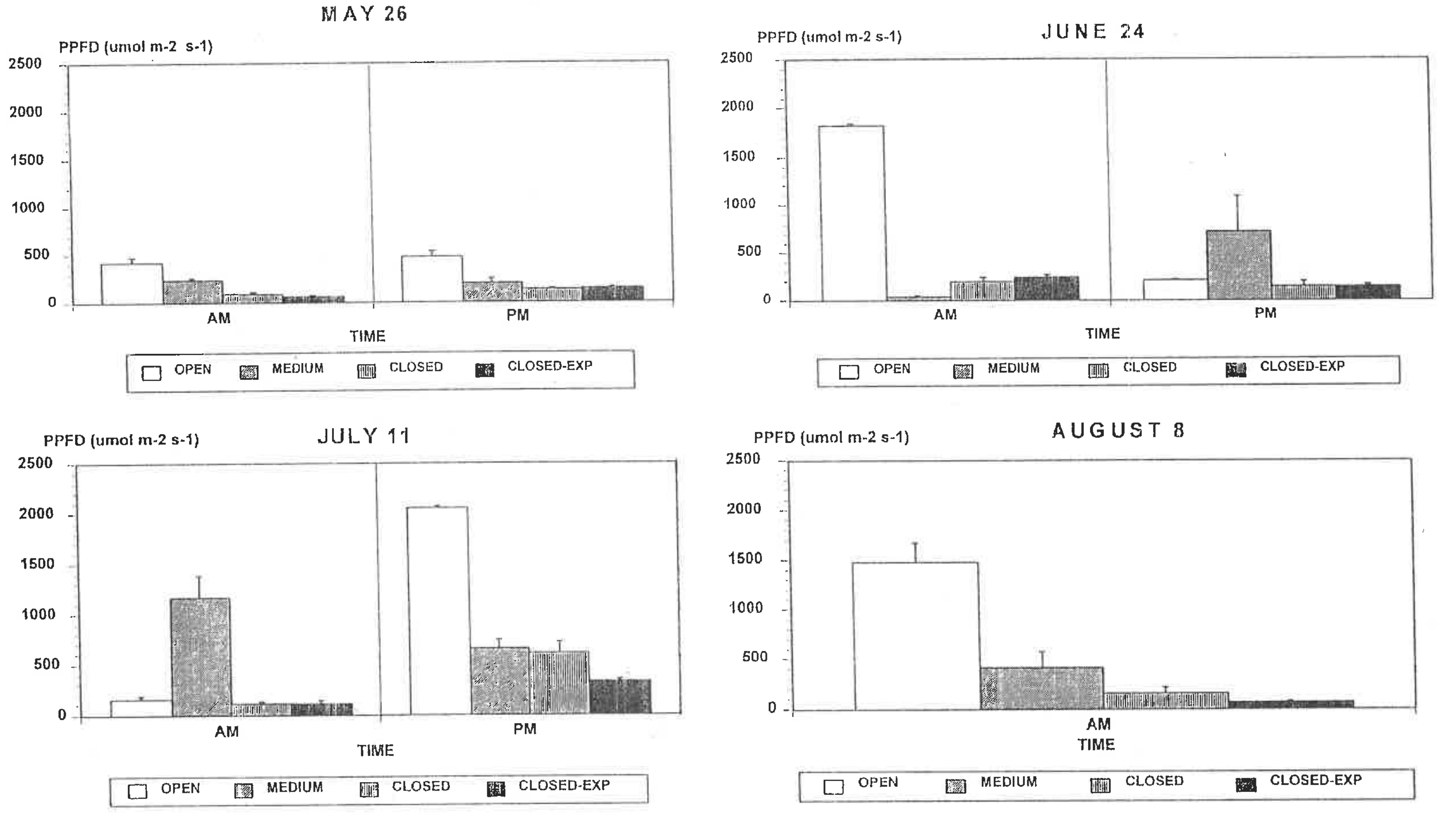

Figure 1. Photosynthetic photon flux density (PPFD) for four environments (open, medium, closed, experimentally closed) in the morning (AM, 0800–1000 hours) and afternoon (PM, 1300–1500 hours) on four dates. Each value is the mean of five measurements. Bars are one standard deviation of the mean.

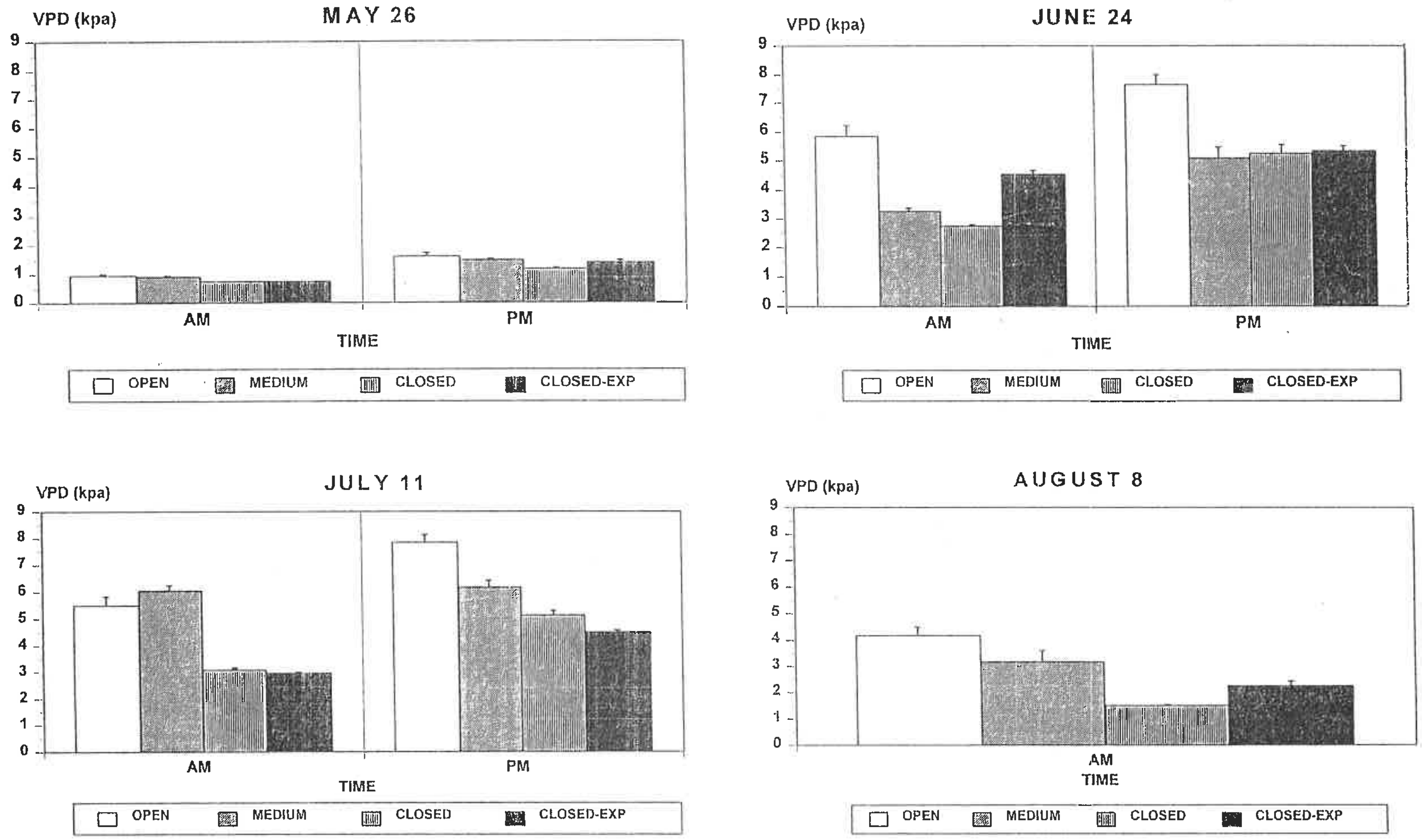

Figure 2. Leaf-to-air vapor pressure deficit (VPD) for four environments (open, medium, closed, experimentally closed) in the morning (AM, 0800–1000 hours) and afternoon (PM, 1300–1500 hours) on four dates. Each value is the mean of five measurements. Bars are one standard deviation of the mean.

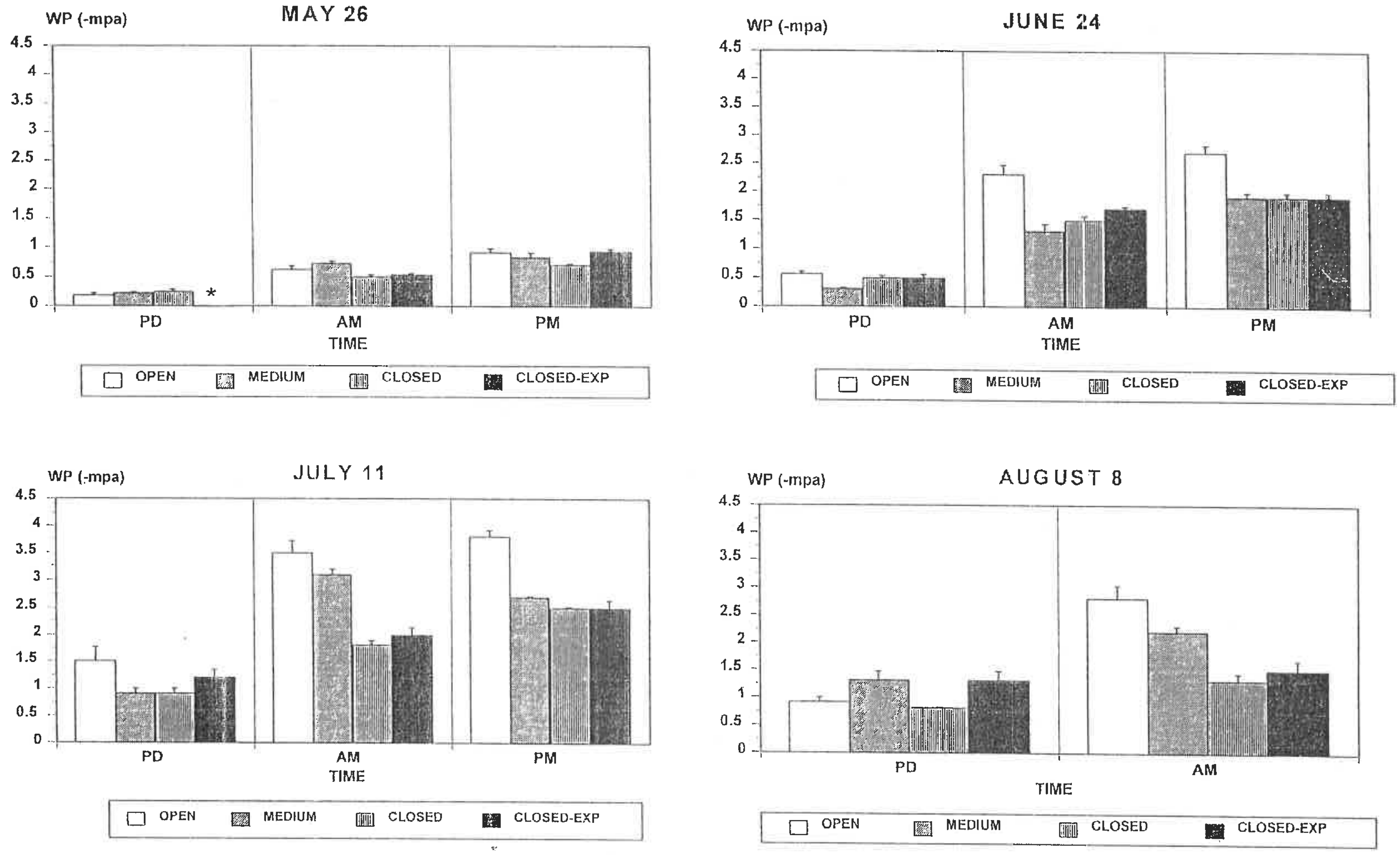

Figure 3. Foliar water potential for four environments (open, medium, closed, experimentally closed) at pre-dawn (PD, 0600 hours), morning (AM, 0800–1000 hours) and afternoon (PM, 1300–1500 hours) on four dates. Each value is the mean of five measurements. Bars are one standard deviation of the mean. The asterisk (*) for May 26 indicates missing data for the experimentally closed treatment.

potential among environments was small at all times of the day, consisting of maximum differences of 0.25 MPa. In June, differences in pre-dawn water potential among environments were again small in magnitude and not statistically significant. However, June AM and PM water potential was significantly lower in open compared with other environments. In July, water potential at all times of the day was significantly lower in open compared with other environments. There were no significant differences in July water potential among medium, closed, and experimentally closed environments except in the AM, where water potential in the medium environment was lower than in the closed and experimentally closed environments. August pre-dawn water potential did not differ significantly among environments, while AM water potential was significantly lower in open and medium compared with closed and experimentally closed environments.

Net photosynthetic rate (leaf area basis) differed widely among dates in the open environment with the highest rates in June and the lowest rates in May and July (Figure 4). In contrast, there was little variation in net photosynthetic rate among dates for the shaded environments. Net photosynthetic rate differed significantly ($p < 0.0001$) among environments in May, June, and August, but not July. On those dates where significant differences among canopy environments occurred, both AM and PM net photosynthetic rates were significantly higher in open versus shaded environments. On most dates, maximum daily values of net photosynthetic rate were greater in medium compared with closed and experimentally closed environments, but these differences were not statistically significant. Differences in net photosynthetic rate between closed and experimentally closed environments were small and not significant on all dates. The time of day that measurements were taken was a significant ($p < 0.04$) source of variation in net photosynthetic rate in June and July, and environment x time was significant ($p = 0.03$) in June. Net photosynthetic rates in both June and July were generally greatest in the AM versus the PM period, the only exceptions being the medium environment in June and the experimentally closed environment in July. Differences in net photosynthetic rate among dates, environments, and measurement times calculated on a leaf dry weight basis (data not shown) were similar to differences calculated on a leaf area basis.

Correlations between net photosynthetic rate and water potential in May, June, and July were not significant (Figure 5). However, in August net photosynthetic rate was significantly and negatively correlated with water potential. In contrast, net photosynthetic rate was significantly and positively correlated with light intensity at the time of measurement in May, June, and August, but not July (Figure 6).

Discussion

Our results support the hypothesis that shading from the *Pinus ponderosa* canopy ameliorates daytime water stress in adult *Clematis*. Morning and afternoon water potentials were consistently lower in open compared with shaded environments in June, July, and August, indicating greater cumulative water stress in the open environment. Differences in morning and afternoon water potentials among environments were predominantly the result of greater transpiration in open versus shaded environments driven by higher leaf-to-air vapor pressure deficit and higher stomatal conductance in open environments (data not shown) rather than differences among environments availability of soil moisture. Pre-dawn water potential is a sensitive measure of soil water availability in the rooting zone (Kozlowski et al. 1991). The similarity of pre-dawn water potential among canopy environments in May and June indicates similar availability of soil water in the rooting zone in early summer when soil moisture is primarily supplied by residual winter and spring precipitation. Differences in pre-dawn water potential among environments in early July suggest lower water availability in open compared with shaded environments following depletion of residual winter precipitation but prior to the start of summer monsoonal rains. In early August following the start of the monsoonal rains, there was again no evidence of differences in availability of soil water among environments. Thus, on most dates availability of soil water in the rooting zone was similar among environments.

Net photosynthetic rate of *Clematis* was more strongly limited by shading than by water stress. Differences in net photosynthesis among environments were strongly related to light intensity in May, June, and August, indicating maximum carbon assimilation in the open environment. In July, the relationship between net photosynthetic rate and light intensity was still positive, but weaker than in other months perhaps due to se-

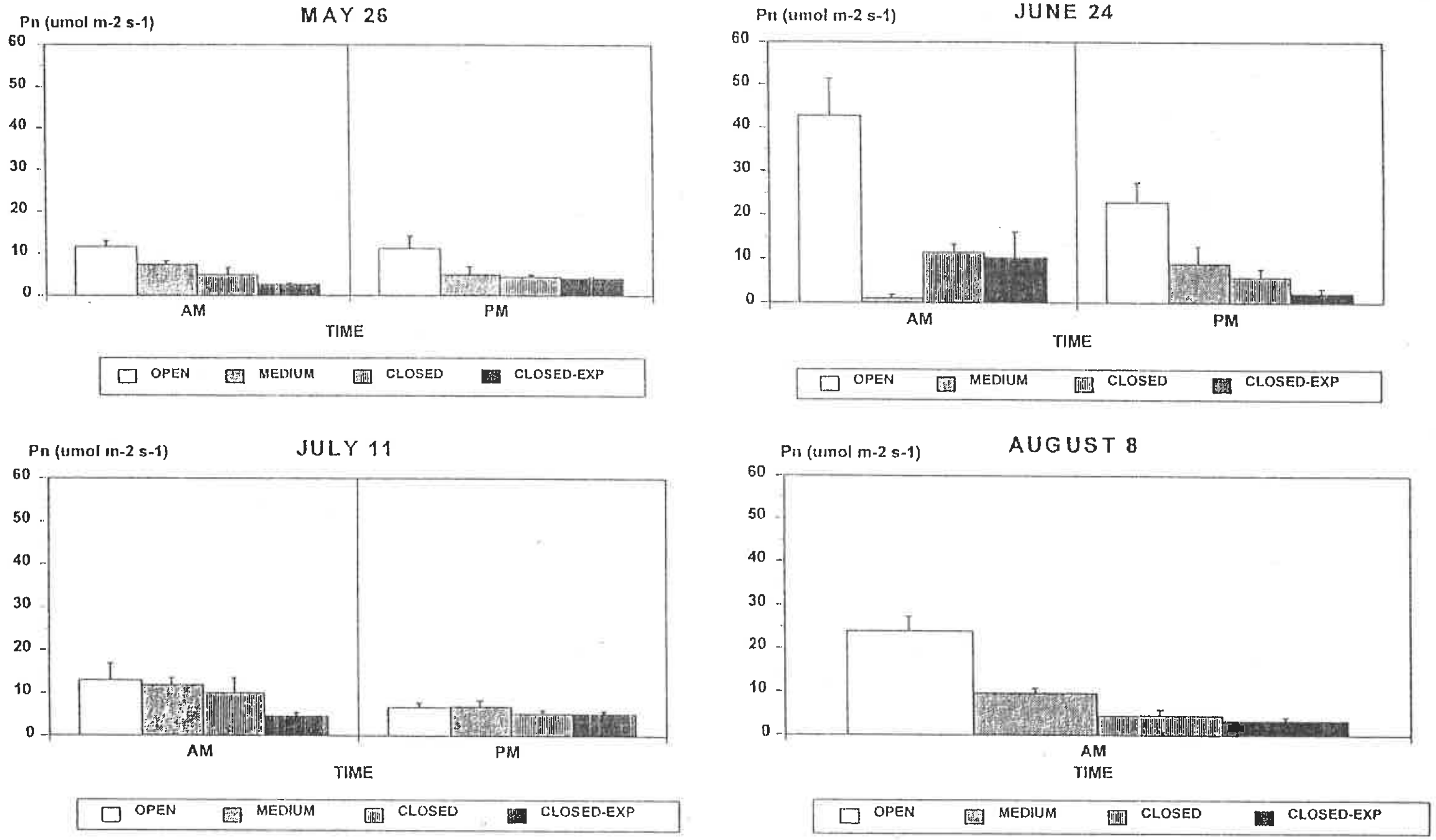

Figure 4. Net photosynthetic rate for four environments (open, medium, closed, experimentally closed) in the morning (AM, 0800–1000 hours) and afternoon (PM, 1300–1500 hours) on four dates. Each value is the mean of five measurements. Bars are one standard deviation of the mean.

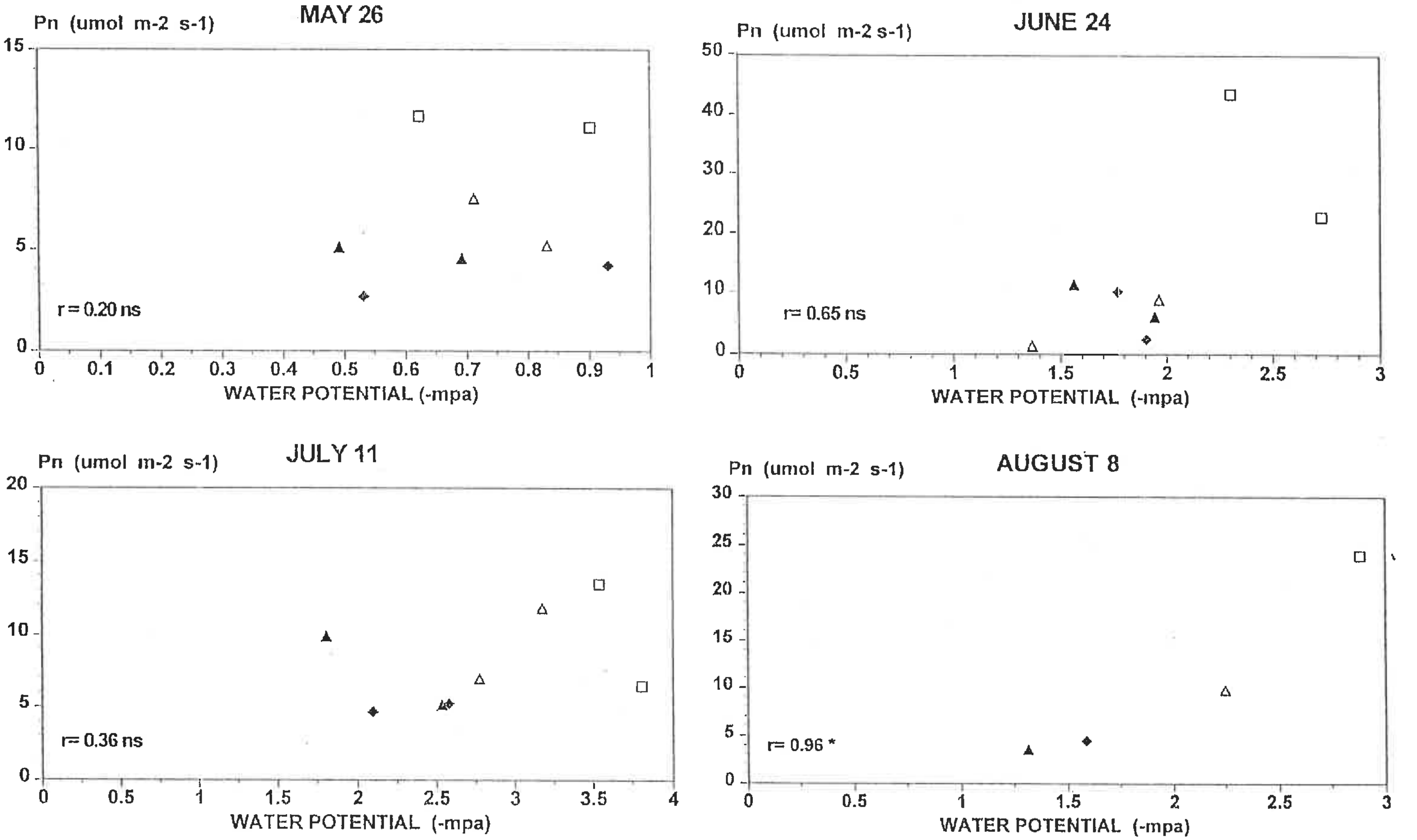

Figure 5. Relationship between net photosynthetic rate and foliar water potential for four environments on four dates. Values for each date and environment are means for morning and afternoon measurements. Environments are coded as: open = white square, medium = white triangle, closed = black triangle, experimentally closed = black diamond.

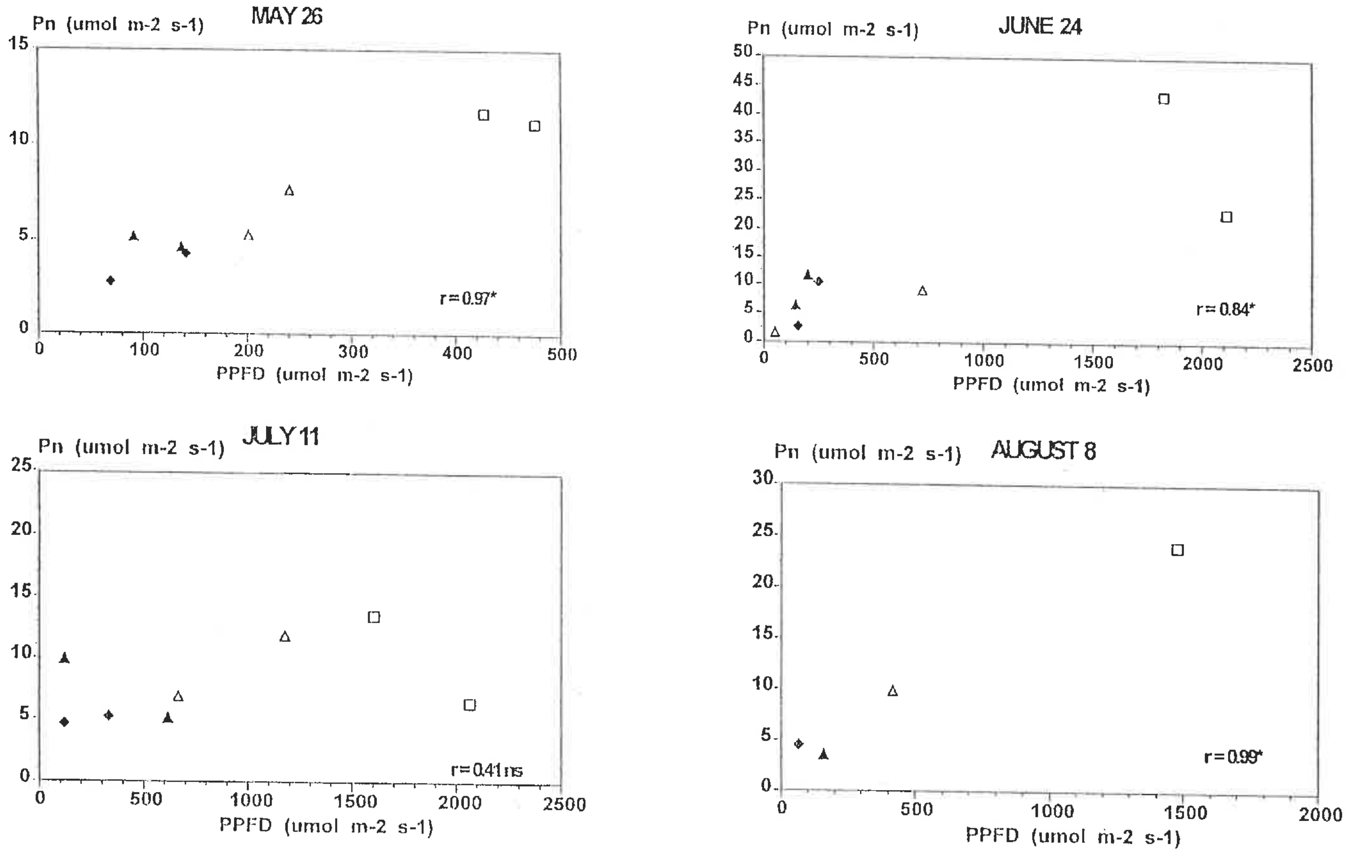

Figure 6. Relationship between net photosynthetic rate and photosynthetic photon flux density (PPFD) for four environments on four dates. Values for each date and environment are means for morning and afternoon measurements. Environments are coded as: open = white square, medium = white triangle, closed = black triangle, experimentally closed = black diamond.

vere daytime water stress in the open environment (water potentials < -3.5 mPa) which limited net photosynthesis in this environment to a greater degree than in the more shaded environments. In May, June, and July, net photosynthetic rate was not significantly related to water stress. The only significant relationship between net photosynthetic rate and water stress occurred in August where high rates of photosynthesis were associated with high levels of water stress (more negative water potentials), which apparently resulted from high transpiration rates associated with high rates of stomatal conductance in the open environment. Adult *Clematis* in northern Arizona are capable of maintaining high rates of net photosynthesis in open environments despite the occurrence of high evaporative demand and severe daytime water stress. This physiological characteristic is likely an adaptation to montane environments of the southwestern United States, which are characterized by high light intensity, low humidity, and prolonged periods of low soil moisture during the growing season.

There was no apparent direct linkage between effects of canopy cover on the *Clematis* net photosynthethic rate measured in this study and reproductive success as measured in previous studies. Although net photosynthesis of adult *Clematis* was highest in open environments, reproduction and population growth are favored by shadier environments. Previous research on *Clematis* in northern Arizona (Maschinski et al. in press) has indicated lower seedling establishment in open versus more shaded environments. Plants growing in environments with average light levels greater than 75 percent full sun suffered from higher bud mortality, lower seed viability, lower seedling establishment, higher mammalian herbivory, and greater exposure to competition from other vegetation than plants growing in more shaded environments (Maschinski et al. in press). For *Clematis*, environments with intermediate levels of canopy cover characterized by frequent sunflecks may offer the best combination of exposure to direct sun, which stimulates photosynthesis but induces daytime water stress, and shading, which depresses photosynthesis but ameliorates daytime water stress through stomatal closure. Our research suggests that timber harvesting activities that completely remove overstory shade will be detrimental to *Clematis* populations in northern Arizona, because of high levels of water stress, which likely damages flowers and young seedlings by desiccation. However, harvesting activities that maintain intermediate levels of shading (i.e., >50% canopy cover), where vegetation receives filtered light with frequent sunflecks, should not be detrimental.

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