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# A test of gas exchange measurements on excised canopy branches of ten tropical tree species

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## Abstract

We studied gas exchange of leaves on branches that had been cut and then re-cut under water to assess the utility of measuring gas exchange on leaves of excised canopy branches. There was large variation between species in their ability to photosynthesize following excision. Some species maintained up to 86.5 % of intact photosynthetic rate 60 min after excision, whereas other species dropped below 40 % of intact photosynthetic rates within 3 min. Three species showed significant reductions in maximum rates of gross photosynthetic rate ($P_G$) on leaves of excised branches relative to intact branches. Excision significantly reduced carboxylation rates ($Vc_{max}$) in four species and electron transport ($J_{max}$) in two species. There were also significant increases in compensation irradiance and reductions of day rates of respiration relative to intact measurements. While gas exchange on excised branches can provide useful measures for canopy species, responses of individual species to branch excision need to be taken into account. Measurements on pre-screened species allow a greater understanding of canopy photosynthesis of large trees when canopy access is not an option.

*Additional key words:* canopy physiology; $CO_2$ response curve; Panama; potometer; species differences in photosynthetic characteristics; specific leaf area; stomatal conductance; tropical forest.

## Introduction

Photosynthetic measurements from upper canopy leaves are useful for understanding carbon balance in large trees, for comparing carbon economies among species, and for modeling biosphere-atmosphere interactions at local and regional scales (Running and Coughlan 1988, Williams *et al.* 1996). Canopy access techniques, such as towers, scaffolds, and construction cranes, have facilitated photosynthetic measurements from terminal leaves of large canopy species (Mulkey *et al.* 1996). But these structures are not available everywhere and there is need for reliable estimates of leaf-level processes in places where it is not practical to build such structures. Researchers have measured gas exchange of canopy leaves by cutting or shooting small branches and re-cutting stems under water to re-establish the xylem water column in the form of a potometer before measurement (Koyama 1981, Reich *et al.* 1995, 1998, Dang *et al.* 1997). Other researchers have measured gas exchange of trees by simply detaching a group of leaves and placing them in a cuvette for immediate measurement (Ginn *et al.* 1991, Gerrish 1992, Samuelson 1998).

Although numerous physiological observations on excised foliage have been published, there is evidence that excision may alter gas exchange measurements in several ways. Even if a stem is double cut, cavitation, changes in xylem pressure, and reductions in hydraulic conductance can result (Boari and Malone 1993, Stahlberg and Cosgrove 1995). These alterations in the xylem stream can influence stomatal responses (Sperry *et al.* 1993, Williamson and Milburn 1995) and inhibit transport of hormones and nutrients, which are important for regulating shoot water potential and stomatal control (Zhang and Davies 1990, Tardieu and Davies 1993). The time scale at which these changes occur is likely to be important in determining the degree of excision-induced effects on gas exchange. However, these responses vary widely across the plant kingdom leaving us with little information regarding the reliability of gas exchange measurements on excised foliage; few studies provide verification of how leaves on excised stems perform (Ginn *et al.* 1991, Dang *et al.* 1997).

The purpose of this study was to compare gas exchange rates measured on excised and intact branches of tropical forest canopy trees. We investigated how measurements of gas exchange and biochemical parameters derived from photosynthetic irradiance and $CO_2$ response curves vary between leaves on excised and intact branches. We also evaluated whether species differ

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in their gas exchange responses to excision, and if there are any characteristics that might be used to predict the degree of excision-induced effects.

## Materials and methods

he study was conducted from two construction cranes operated by the Smithsonian Tropical Research Institute (STRI) in the Republic of Panama between May–July 1999 and June–July 2001. Each crane is equipped with a gondola suspended by cables from a rotating boom that allows coverage of approximately 0.82 ha of forest. The first crane is located in Parque Metropolitano (PM), a seasonally dry forest on the edge of Panama City that receives approximately 1 800 mm of precipitation annually with a distinct dry season between December and April. The second crane is located at Fort Sherman (FS), on the Caribbean side of the Panamanian Isthmus in forest that receives appro-ximately 3 100 mm of precipitation annually with a shorter and less intense dry season.

Table 1. Initial values of intact net $CO_2$ assimilation rate ($P_N$), stomatal conductance ($g_s$), and specific leaf area (SLA) ± 1 S.D. for canopy leaves of 10 tropical tree species from the Republic of Panama. $n$ = 3-5.

| | Species | $P_N$ [μmol $m^{-2}$ $s^{-1}$] | $g_s$ [mmol $m^{-2}$ $s^{-1}$] | SLA [$m^2$ $kg^{-1}$] |
|---|---|---|---|---|
| Parque Metropolitano | *Anacardium excelsum* | 7.0 ± 2.0 | 129.7 ± 37 | 9.31 ± 0.68 |
| | *Luehea seemannii* | 14.8 ± 1.6 | 592.2 ± 140 | 7.95 ± 1.19 |
| | *Pseudobombax septenatum* | 12.4 ± 1.7 | 347.0 ± 57 | 11.86 ± 0.77 |
| Fort Sherman | *Apeiba membranacea* | 16.1 ± 1.3 | 659.7 ± 103 | 12.48 ± 0.96 |
| | *Aspidosperma cruenta* | 14.2 ± 0.7 | 387.3 ± 68 | 7.44 ± 0.73 |
| | *Brosimum utile* | 10.4 ± 1.6 | 298.2 ± 83 | 9.12 ± 0.42 |
| | *Jacaranda copaia* | 14.6 ± 2.5 | 538.0 ± 212 | 9.66 ± 0.64 |
| | *Manilkara bidentata* | 11.8 ± 3.4 | 199.3 ± 83 | 4.94 ± 0.17 |
| | *Simarouba amara* | 15.7 ± 1.7 | 443.8 ± 113 | 6.81 ± 0.54 |
| | *Vochysia ferruginea* | 15.2 ± 0.8 | 670.2 ± 83 | 8.95 ± 0.97 |

Ten tree species from nine families, representing a variety of leaf morphology and gas exchange rates, were investigated (Table 1). Net $CO_2$ assimilation ($P_N$) and stomatal conductance ($g_s$) were measured with an infrared gas analyzer (model *6400*, *Li-Cor*, Lincoln, NE, USA) on leaves with full sun exposure from branches of 3-5 individuals. All photosynthetic measurements were taken between 07:00 and 10:30 h. One gas exchange measurement was taken on a newly formed mature leaf on an intact branch at ambient temperature (28–33 ºC), ambient relative humidity (70–80 %), 37.26 Pa $CO_2$ (equivalent to 370 μmol $mol^{-1}$, slightly higher than ambient), and 1 500 μmol $m^{-2}$ $s^{-1}$ photosynthetic photon flux density (PFD) provided by a red blue radiation source (model *6400-02B #SI-710*, *Li-Cor*). The segment of branch was then excised 100 cm from the measured leaf and immediately shortened to 50 cm by re-cutting under water to re-establish the xylem water column. After excision, gas exchange measurements were taken within 3 min and then several times up to 60 min on the excised branch and on a control leaf on an intact branch.

The response of $P_N$ to PFD was measured on newly formed mature canopy leaves from three branches of three individuals for six species at a range of PFD from 0 to 1 500 μmol $m^{-2}$ $s^{-1}$, 37.26 Pa $CO_2$, ambient relative humidity, and ambient temperature. We first measured $P_N$ at 1 500 μmol $m^{-2}$ $s^{-1}$ PFD, then irradiance was decreased in a stepwise fashion for a total of 10 measurement points down to 0 μmol $m^{-2}$ $s^{-1}$. After irradiance response curves, branches were immediately re-cut under water as described above and an irradiance response curve on a leaf of similar age adjacent to the original leaf was repeated in the gondola with the stem in a potometer. Leaves on the cut stem were maintained in sunlight to prevent stomatal closure as a result of low photon availability in the gondola. Parameters for irradiance response curves were fit to a nonrectangular hyperbola (Sims and Pearcy 1991), allowing calculation of compensation irradiance ($I_c$), apparent quantum yield on the basis of incident photons ($\Phi$), photon-saturated rate of gross $CO_2$ assimilation at saturating irradiance ($P_G$), and dark respiration rate ($R_D$).

The effect of branch excision on $CO_2$ response curves was measured on branches of three individuals from six species. We conducted measurements on intact branches by first inducing the leaf with 1 700 μmol $m^{-2}$ $s^{-1}$ PFD and 37.26 Pa $CO_2$. We then took measurements at 0 Pa $CO_2$ and gradually increased $CO_2$ concentration over a total of 12 points to a final concentration of 151 Pa. We then cut and re-cut the branch as described above and a second $CO_2$ response curve was conducted on a leaf of similar age adjacent to the original leaf. Parameters for $CO_2$ response curves were modeled by two equations that describe $CO_2$-limited and ribulose-1,5-bisphosphate carboxylase/oxygenase-limited rates of photosynthesis and allow calculation of maximum rate of electron transport ($J_{max}$), rate of $CO_2$ assimilation at saturating $P_i$ ($Vc_{max}$), and day rates of respiration ($R_{day}$) (Caemmerer and Farquhar 1981, Lambers *et al.* 1998). Analysis of variance (ANOVA) and Duncan's multiple range tests were used to assess the effect of excision on parameters calculated from irradiance and $CO_2$ response curves.

## Results and discussion

The response of gas exchange to branch excision varied widely among the ten species studied. After excision, all species showed some reduction in $P_N$ relative to average intact rates within 3 min (Fig. 1). After 10 min, most species showed fewer fluctuations and appeared to stabilize for the remaining 60 min. Excision reduced $P_N$ by 14–87 % at Parque Metropolitano and by 21–77 % at Fort Sherman. *Anacardium excelsum* showed a 41 % reduction in $P_N$ after 60 min in control leaves, making it difficult to distinguish effects of excision from leaf handling during photosynthetic measurements in this species. However, in the other nine species studied, control leaves varied ±12 % of average intact photosynthetic rates. Sixty minutes after excision, $g_s$ was 7–95 % lower than intact values with similar species ranks and patterns as shown for $P_N$ in Fig. 1. In general, responses were species-specific with some species maintaining gas exchange rates near control values for up to 60 min. These data indicate that $P_N$ and $g_s$ respond similarly to branch excision, that these responses are highly species specific, and that gas exchange measurements taken <10 min after cutting may minimize excision-induced effects.

The effect of branch excision on photosynthetic irradiance response curves also varied among species. In *Aspidosperma cruenta*, *Brosimum utile*, and *Simarouba amara*, $P_G$ was significantly lower on excised branches than on intact branches (Fig. 2). We found $I_c$ to be significantly higher on excised branches than on intact branches in these same three species ($F = 3.07$; $p<0.05$). Branch excision had a nearly significant effect on $R_D$ ($F = 2.50$; $p = 0.06$), but in some species this effect was a reduction and in other cases, $R_D$ increased in leaves of excised branches. There were no significant differences in $\Phi$ between leaves from intact and excised branches within species ($F = 0.83$; $p>0.5$). The effects of excision on irradiance response curves reinforce the observation that species respond differently to branch excision. The three species showing significant reductions in $P_G$ after excision produce latex or resin in the stem (Croat 1978, Santiago 2003). Wounding stimulates latex and resin production that can physically clog xylem, preventing water loss and pathogen attack (Kramer and Kozlowski 1979). Our results suggest that if latex or resin leak in the vicinity of the cut stem section in a potometer, it could clog

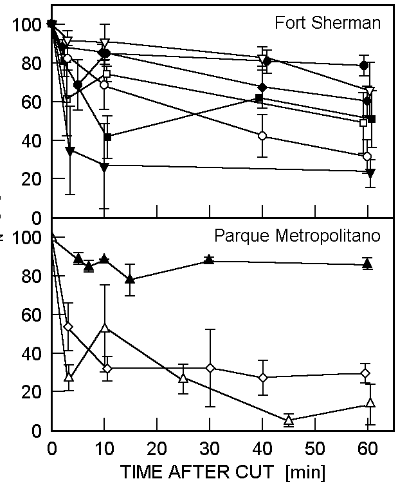

Fig. 1. Percent of intact net photosynthetic rate ($P_N$) of 3-5 canopy leaves from excised branches at two canopy crane sites: Fort Sherman and Parque Metropolitano, Republic of Panama. Species at Fort Sherman: (●) *Apeiba membranacea*, (○) *Aspidosperma cruenta,* (▼) *Brosimum utile*, (∇) *Jacaranda copaia*, (■) *Manilkara bidentata*, (□) *Simarouba amara,* and (♦) *Vochysia ferruginea*. Species at Parque Metropolitano: (◊) *Anacardium excelsum*, (▲) *Luehea seemannii*, and (Δ) *Pseudobombax septenatum*. Means ± 1 SE. $n$ = 3-5.

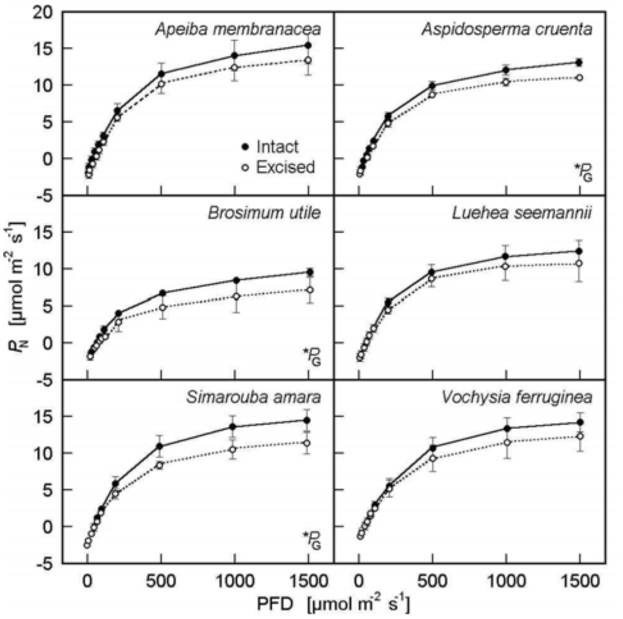

Fig. 2. Curves of net $CO_2$ assimilation ($P_N$) as a function of PFD for leaves of intact and excised canopy branches. Means ± 1SE. *Significant difference ($p<0.05$; $n = 3$) in gross photosynthetic rate ($P_G$) between excised and intact branches.

the xylem, inhibiting transpiration and reducing gas exchange. Therefore, the presence of latex, resin, or other plant traits related to physiology or phylogeny may represent indicators for predicting the response of gas exchange to branch excision.

Branch excision reduced $Vc_{\max}$ in *Apeiba membranacea, Luehea seemannii*, *A. cruenta*, and *S. amara* (Fig. 3). Excision changed the general shape of the $CO_2$ response curve in *A. cruenta* and *S. amara*, whereas in *A. membranacea* and *L. seemannii* lower maxima were the main effect. Additionally, $J_{\max}$ was significantly lower in excised than intact branches of *L. seemannii* and *S. amara* (Fig. 3), and $R_{day}$, calculated from $CO_2$ response curves, was significantly lower in excised branches of *B. utile, L. seemannii,* and *S. amara* ($F = 13.3$; $p<0.0001$). In all cases, $R_{day}$ was greater in intact than excised branches. Hence $CO_2$ response curves were more sensitive to excision than irradiance response curves. In general, reduced $P_G$, $J_{\max}$, $Vc_{\max}$, and $R_{day}$, and increased $I_c$ suggest that branch excision reduces biochemical activity within the leaf. Further studies may address whether changes in leaf water potential, the transport of hormones and nutrients, or other causes drive the observed species-specific responses to branch excision.

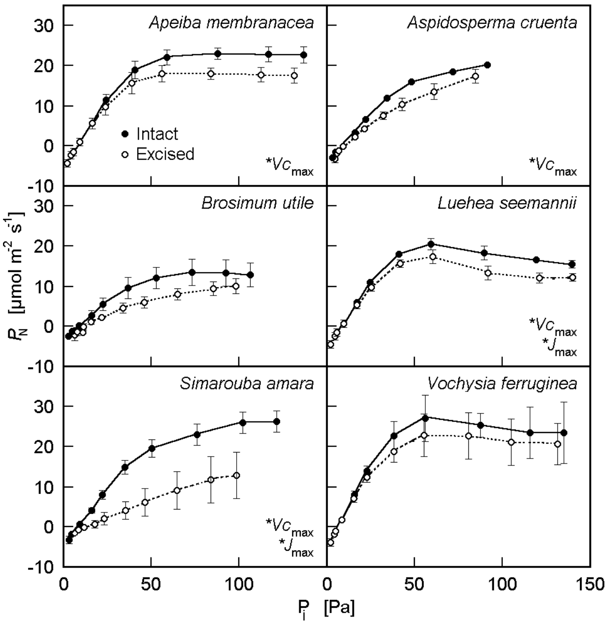

Fig. 3. Curves of net $CO_2$ assimilation ($P_N$) as a function of internal $CO_2$ partial pressure ($P_i$) for leaves of intact and excised canopy branches. Means ± 1 SE. *Significant differences ($P<0.05$; $n = 3$) in $J_{\max}$ and $Vc_{\max}$ between excised and intact branches.

While gas exchange on excised branches can provide useful measures for canopy species, understanding individual species responses to branch excision can greatly improve reliability of data. Investigations of responses of individual species, perhaps through trial studies, may allow identification of species that maintain photosynthetic rates similar to intact values after excision. We emphasize that approximately half of the species studied showed no statistically significant reduction of $P_G$, and that species traits, such as the presence of latex of resin, may allow prediction of physiological responses following branch excision across broader ranges of species. Irradiance response curves or point measurements at saturating irradiance performed quickly after excision represent the most reliable estimates of photosynthetic gas exchange. Careful measurements, performed on pre-screened species, allow a greater understanding of canopy photosynthesis of large trees when canopy access is not an option.

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