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The contribution of respiration in tree stems to the Dole Effect

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Abstract. Understanding the variability and the current value of the Dole Effect, which has been used to infer past changes in biospheric productivity, requires accurate information on the isotopic discrimination associated with respiratory oxygen consumption in each of the biosphere components. Respiration in tree stems is an important component of the land carbon cycle. Here we measured, for the first time, the discrimination associated with tree stem oxygen uptake. The measurements included tropical forest trees, which are major contributors to the global fluxes of carbon and oxygen. We found discrimination in the range of 12.6–21.5‰, indicating both diffusion limitation, resulting in O₂ discrimination values below 20‰, and alternative oxidase respiration, which resulted in discrimination values greater than 20‰. Discrimination varied seasonally, between and within tree species. Calculations based on these results show that variability in woody plants discrimination can result in significant variations in the global Dole Effect.

2011); and the discrimination against the heavier isotope in respiratory oxygen consumption (Lane and Dole, 1956; Guy et al., 1989, 1993). The Dole Effect was suggested to be a useful tracer for paleo-changes in the ratio of ocean to land productivity (Bender et al., 1994; Blunier et al., 2002). However, this use is based on the assumption of significantly different isotopic effects of the land and the oceans. New findings, including indications for significant discrimination during marine photosynthesis and the effect of diffusion in soils on the effective respiratory discrimination, have resulted in a decreased estimated difference between the terrestrial and marine Dole Effects, making it easier to explain why there have not been large shifts in the Dole Effect between glacial and interglacial times (Luz and Barkan, 2011).

As shown by previous studies (Angert et al., 2001, 2003; Angert and Luz, 2001), when the diffusion of O₂ from the atmosphere to the consumption site inside roots and soil aggregates is restricted, the effective discrimination of the soil system depends not only on the intrinsic discrimination in the respiration processes, but also on the discrimination in diffusion, and on the internal oxygen concentration at the consumption site. This effect of diffusion on the discrimination in O₂ uptake is similar to the well known isotopic effect which takes place in CO₂ diffusion and uptake in leaves (Farquhar et al., 1982). Overall, the effect of diffusion tends to lower the effective discrimination of O₂ uptake in soils (Angert et al., 2003).

However, observations in temperate and boreal forest soils demonstrated that a second process must also act to explain the observed $\delta^{18}\text{O-O}_2$ in soil pore space. This process is

1 Introduction

The Dole Effect is defined as the difference between the average isotopic composition of oxygen in seawater (H₂O), and the oxygen in atmospheric O₂. The magnitude of the Dole effect depends mainly on three factors: the isotopic composition of leaf water from which O₂ is produced on land (Farquhar et al., 1993; Gillon and Yakir, 2001), the possible isotopic discrimination during release of O₂ from H₂O during photosynthesis (Eisenstadt et al., 2010; Luz and Barkan,

respiration through the alternative oxidase pathway (AOX). The discrimination of AOX respiration is $\sim 30\%$, which is considerably higher than the $\sim 20\%$ discrimination in “normal” dark respiration through the cytochrome oxidase pathway (COX) (Ribas-Carbo et al., 1995). High discrimination values ($22.5 \pm 3.6\%$) were found in boreal soils (Angert et al., 2003), and can be explained by a large fraction of respiration which goes through the AOX in plant roots.

The emission of CO_2 from aboveground woody tissues amounts to $\sim 16\%$ of the forest annual gross photosynthesis flux (Litton et al., 2007; Ryan et al., 1997; Waring et al., 1998) while belowground woody tissues contribute a similar amount. As a result, the discrimination in woody tissue O_2 uptake may have a significant impact on the global contribution of land to the Dole Effect. To date there have been no measurements of O_2 isotope discrimination in tree wood respiration processes. Here we report the first measurements based on stem O_2 influx for 6 tree species, representing 4 different plant families, including trees from tropical forests that contribute $\sim 40\%$ of global land primary production (Beer et al., 2010). Our results suggest that, as with soil respiration, both the effects of diffusion on one hand, and AOX activity on the other, control the isotopic effects in stem O_2 uptake.

2 Methods

2.1 Sites and trees

Trees from two sites were sampled. The first site was the Givat-Ram campus of the Hebrew University of Jerusalem (HUJI, $31^\circ 46' 15'' \text{N}$ $35^\circ 11' 51'' \text{E}$). At this site we have performed experiments on the following trees: one Apple (*Malus domestica*), one Stone Pine (*Pinus pinea* L.), and one Aleppo Pine (*Pinus halepensis* Mill.). Experiments at this site were conducted from April 2010 to March 2011. The second site was the UNAP site, located at the Center for Research and Forest Learning (CIEFOR) of the National University of the Peruvian Amazon (UNAP) in the community of Puerto Almendras, which is located 16 km southwest of the city of Iquitos, Peru. CIEFOR is centered over $3^\circ 49' 53'' \text{N}$, $73^\circ 22' 28'' \text{W}$, encompasses a forested area of 1300 ha and belongs to the Faculty of Forest Engineering (FCF)-UNAP. For the base period 1971–2000 the mean annual rainfall is ~ 3000 mm (<http://www.senamhi.gob.pe/>), and the maximum, minimum, and average temperatures are 26.3°C , 25.9°C , and 25.2°C , respectively (Brohan et al., 2006). All the experiments in this study were conducted on a total of nine trees from the following species (three each): *Tachigali paniculata* (Tangarana) and *Hymenolobium* sp. (Mari-Mari) from the Fabaceae family, and *Simarourea amara* (Marupa) from the Simaroubaceae family, with a typical wood density value of 0.53, 0.65, and 0.35 g ml^{-1} , respectively (Chambers et al., 2004). The experiments in the

UNAP site were conducted during the local wet season, from April to May 2011.

2.2 Stem chambers

The stem chambers used at the HUJI site are described in Angert and Sherer (2011). Each chamber was constructed from two rectangular clear Perspex parts: (1) a frame base equipped with closed-cell foam on the stem side, and (2) a lid equipped with plastic connectors for sampling and a 60 ml syringe directly connected to it to allow decreasing the system's volume while taking an air sample. The bottom part of the syringe was sawed off to allow good diffusive mixing with the rest of the chamber. The chamber was sealed to the stem by hot glue. For installation on trees where rough bark made it difficult to make an air-tight seal with the chamber base, we first removed some bark, and then smoothed the surface with a file, while being careful not to damage the cambium. In case of smooth-barked trees, we only removed loose bark and lichen before installing the chamber base. After closing the lid, we checked the seal by pulling the piston of the bottomless syringe attached to the lid, and checking for resistance to the pull. This resistance indicates that air could only enter the chamber through small pores in the bark, and hence, in the absence of strong winds (which were avoided during our field campaigns), the mixing between the chambers and the atmosphere was dominated by diffusion. After incubation of 48 to 141 h, the samples of the air in the chamber were collected in pre-evacuated ~ 3.6 ml glass flasks with a Louwers-Hapert™ O-ring valve. Before sampling, the dead volume in the tubing and flasks' necks was purged with 30 ml of air from the chamber. In addition to the tree stem experiments, we also set up a blank experiment, to test the possible effect of water vapor diffusion from a stem to the atmosphere. In this experiment we attached a stem chamber to a wood board (3.5 cm thick) that was soaked in water overnight. The board and the chamber were then positioned next to the Apple tree (in August 2012), and the chamber air was sampled after 24 h.

The chambers used in the UNAP site were based on the design reported in Ubierna et al. (2009), and made from polypropylene (PP) tube T-pieces (OD 11 cm, Ostendorf HTRE DN 110) that are equipped with a threaded lid to close one end. The other two ends were welded shut with PP disks. These completely closed T-pieces were then cut longitudinally, thus removing a segment of the tube opposite to the threaded lid, resulting in an opening along the whole length of the tubing (27.2 cm) and 7.0 cm wide. The chambers were fit to the shape of the tree stem at the exact spot of installation, and sealed to the stem by hot glue. Leak testing was performed by measuring the chamber CO_2 concentration while blowing respiratory air through a piece of tubing on all possibly leaky spots. The CO_2 was monitored with an Infra Red Gas Analyzer (IRGA, LI-820 from Li-Cor). Gas from the chamber was pumped through a water

trap filled with Drierite into the IRGA at a constant flow rate of $\sim 600 \text{ ml min}^{-1}$, and then pumped back into the chamber. The removable chamber lids were equipped with connectors that allowed the 3.6 ml flasks to be directly connected once the lid was sealed. The flask valves were left open to allow O_2 to diffuse freely between the sampling flask and the chamber headspace. These flasks were left open and attached for 10 days to ensure that the chamber air reached steady-state between the rate of “leakage” of O_2 from the atmosphere, and the rate of consumption of O_2 by the stem (see next section). The flasks were then closed and shipped for analysis. The atmospheric pressure monitored in nearby metrological stations was also recorded. Temperature gradients between the stem chamber and the atmosphere can be another source of pressures gradient. In the experiments in Jerusalem this could induce some mass flow, but in the tropical forest experiments the diurnal temperature cycle is small, and this effect was probably negligible. At both sites, the chambers were attached at heights of ~ 1.6 to ~ 2 m above the ground.

2.3 Analytical methods

Sample preparation and mass spectrometry were according to Barkan and Luz (2003). The preparation of the sample included cryogenic removal of water vapor and CO_2 , and chromatographic separation of N_2 by a fully automated system. Elimination of N_2 prevented the need for correction for the effect of N_2 interference in the ion source of the mass spectrometer. The oxygen concentrations were calculated from the ratio of O_2 to Ar (expressed as $\delta\text{O}_2/\text{Ar}$), under the assumption of constant Ar concentration. All measurements were performed on a Finnigan-MAT Delta-Plus (Thermo Scientific, Waltham, MA, USA) dual-inlet mass spectrometer. The precision in $\delta\text{O}_2/\text{Ar}$ was 1‰ (which translates to a precision of 0.02 % in O_2 concentration), and the precision in $\delta^{18}\text{O}$ determination was 0.03‰. All the results are presented with respect to atmospheric air standard (Barkan and Luz, 2003).

2.4 Models for stem and chamber gases

Estimating the discrimination associated with stem O_2 uptake from the $\delta^{18}\text{O}$ and $[\text{O}_2]$ data requires some simple modeling. Our 1-box analytical model follows a model originally developed for soils (Angert et al., 2001, 2003). In our model, a box represents the chamber and the top layer of the stem, which are assumed to have the same O_2 concentration and isotopic composition. The O_2 in the box is assumed to be in steady-state. This steady-state results from a balance between O_2 diffusion into the box through the bark and outer layer of the stem, and O_2 consumption by respiration in the tissues under the chamber.

We assume that advective transport of O_2 dissolved in xylem water transiting the area enclosed by the chamber, as well as possible dissolution or exsolution of O_2 in this wa-

ter, can be neglected compared to respiration consumption of O_2 . This assumption is reasonable because of the relatively low solubility of O_2 in water, which dictates relatively small rates of transport in the xylem water. Even if we assume that the water that arrives at the base of the stem has zero oxygen, this water, at the maximum, could take up dissolved O_2 until it is in equilibrium with atmospheric air, or a concentration of roughly 0.25 mmol l^{-1} . If we assume, for example, a tree with a stem diameter of 0.5 m, respiration rate of $200 \text{ mg C m}^{-2} \text{ h}^{-1}$, and xylem water flux of 500 l d^{-1} , the removal of O_2 by dissolution will amount to 10 % of the oxygen consumed by respiration up to the height of 1 m. However, above this height the water will be saturated and will not be able to take up more oxygen. We assume that in a more realistic case, the water arrives from the roots close to equilibration with the stem O_2 and can take up or lose only small amounts of oxygen compared to respiration fluxes. This will have also only small effect on the O_2 isotopes. This also means that O_2 produced by cortical photosynthesis outside of the chamber will have negligible effect on the chamber $\delta^{18}\text{O}$. We will also neglect here the possible effect of thermal diffusion, under the assumption that the temperatures at the stem surface and inside it are similar.

Hence, the temporal change in O_2 concentrations in the chamber is given by

$$\frac{d[\text{O}_2]_c}{dt} = I - O - R, \quad (1)$$

where the “c” subscript designates the chamber, I is the incoming diffusion flux, O is the outgoing diffusion flux, and R is the consumption flux in the soil. The isotopic composition of the O_2 in the chamber will depend on the balance between the entrance of atmospheric O_2 , which will be affected by discrimination in diffusion, which favors the entrance of light ($^{18}\text{O}/^{16}\text{O}$) oxygen into the chamber headspace, and uptake of O_2 by the stem, which removes light oxygen from the chamber headspace. Temporal changes in the $\delta^{18}\text{O}$ value of the chamber O_2 ($\delta^{18}\text{O}_c$) can be approximated by

$$\frac{d([\text{O}_2]_c \cdot \delta^{18}\text{O}_c)}{dt} = I \cdot (\delta^{18}\text{O}_{\text{atm}} - D_{\text{diff}}) - O \cdot (\delta^{18}\text{O}_c - D_{\text{diff}}) - R \cdot (\delta^{18}\text{O}_c - D_{\text{stem}}), \quad (2)$$

where D_{diff} is the ^{18}O discrimination in diffusion in air, and $\delta^{18}\text{O}_c$ and $\delta^{18}\text{O}_{\text{atm}}$ are the $\delta^{18}\text{O}$ values of the chamber and atmospheric O_2 respectively. D_{stem} is the overall ^{18}O discrimination of the O_2 uptake by the stem. The D_{stem} parameter integrates the combined effects of the diffusion in gas phase in the stem, possible diffusion in liquid phase to the consumption site within living cells, and the biochemical uptake by both COX and AOX. Since we are interested in the change in $\delta^{18}\text{O}$ relative to atmospheric oxygen (our primary standard), $\delta^{18}\text{O}_{\text{atm}}$ is zero and can be omitted, and in steady-state we obtain (Angert et al., 2001; Farquhar et al., 1982) :

$$D_{\text{stem}} = \frac{\delta^{18}\text{O}_c}{(1 - [\text{O}_2]_c/20.95\%)} + 14.1\%, \quad (3)$$

where the values of 20.95 % and 14.1 ‰ are the O₂ concentrations in air, and the discrimination associated with O₂ diffusion in air, respectively.

Another way to represent the balance between diffusion and uptake is by a numerical model. Our 1-box numerical model is based on the same box and fluxes as the analytical model above. The main difference is that steady-state is not assumed between the box and the atmosphere. Rather, the model is initialized to start with atmospheric air in the box, and the changes in the O₂ and CO₂ are solved by a finite-difference approach. An example of a model run (with $D = 14.1$ ‰) appears in Fig. 1. As the simpler analytical model predicts, discrimination of 14.1 ‰ is associated with a $\delta^{18}\text{O}$ value of 0 ‰ (i.e. identical to the atmosphere) in steady-state. However, the time-resolving model shows that $\delta^{18}\text{O}$ overshoots to values above 1 ‰ before reaching steady-state. Hence, using the analytical model for a sample taken before the chamber reached steady-state will yield an erroneous estimate of the discrimination value. With the arbitrary conductance used in this example, the steady-state values in isotopic composition are achieved in ~ 30 h. This isotopic equilibration time is much longer than that required for concentrations to come into equilibration, as has been observed in other systems. Based on previous experiments (Angert and Sherer, 2011) we have estimated that the time needed for the isotopic composition in the stem chamber to reach steady-state is about 24 to 48 h. Consequently, we calculated here the discrimination by Eq. (1), only from chambers' experiments lasting at least 48 h.

The numerical model was also refined to include additional possible effects. One of these effects is the “water vapor flux fractionation effect”, which is driven by the diffusion of water vapor (Severinghaus et al., 1996). If a gradient in the concentration of water vapor is present in the outer bark layer, this effect can have some impact on the isotopic composition. A somewhat similar effect can be introduced if the rate of oxygen uptake by the stem exceeds the rate of CO₂ emission. This would be accompanied by a reduction in pressure with a consequent mass flow of atmospheric air into the box. This will not only bring in O₂, but also N₂ and Ar. As a result, N₂ and Ar concentrations in the stem will increase, and the O₂/Ar ratio will not simply indicate O₂ concentrations. We thus included all the relevant gases in our model, and assumed that any pressure gradient will be compensated by mass flow. Using this version of the model we found that these effects are small and tend to cancel out, so that even if we assume that the outer bark has a relative humidity as low as 60 %, or the CO₂/O₂ fluxes have a ratio of 0.5, the error in the estimation of D by Eq. (3) will be < 0.3 ‰, and thus can be neglected. To further confirm the conclusion that the water vapor effect is negligible, we have performed the blank experiment described above.

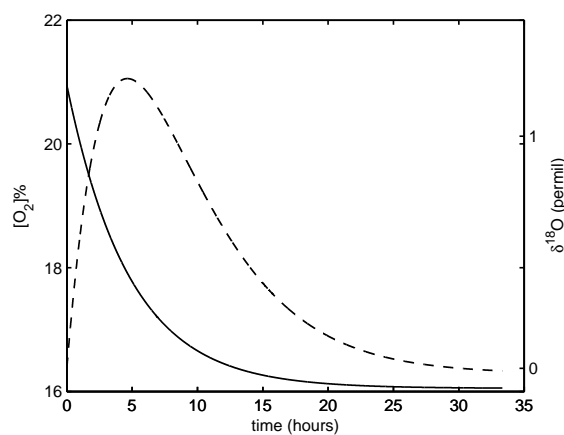


Fig. 1. Modelled O₂ concentrations (solid line) and $\delta^{18}\text{O}$ values (dashed line, versus atmospheric air) in the stem chamber, for a model run with $D_{\text{stem}} = 14$ ‰.

3 Results

In the air from the blank experiment, the $\delta^{18}\text{O}$ and $\delta\text{O}_2/\text{Ar}$ were -0.07 ‰ and -6 ‰, respectively. The results of all the stem chambers experiments are summarized in Table 1. The discrimination values (D) ranged from 12.0 ‰ to 21.5 ‰. Discrimination in the range of 14–16 ‰ was the most common and was found in 9 out of the 21 experiments. Discrimination below 16.5 was found in almost all the Apple tree experiments, excluding the one conducted shortly after bud burst in April 2010, in which the discrimination was 21.5 ‰. The Mari-Mari trees' discrimination was 15.2–15.3 ‰, while the Tanagrana trees' range was 16.6–18.1 ‰, and the range for the Marupa trees was the widest: 15.2–19.8 ‰.

4 Discussion

The estimate of the discrimination by Eq. (3) is based on the assumption that the gas transport to the chamber is dominated by gas-phase diffusion. In other words, we assume that O₂ did not enter the chambers by mass flow. Entrance of O₂ by mass flow is with no isotopic discrimination, and hence using the parameter of 14.1 ‰ in Eq. (3) will bias D_{stem} toward higher values, if the assumption of dominating diffusion gas exchange is not valid. However, this assumption is supported by two tests we have made. First, we have successfully tested the chambers for leaks (see Sect. 2). Second, we have conducted an experiment on the Apple tree especially to test the assumption of dominating gas-phase diffusion. In this test, we have flushed the chamber at the beginning of the experiment with N₂ gas. In this condition, the initial gas exchange between the chamber and the atmosphere brings in O₂ to the chamber. Oxygen entering by mass flow will enter with the same isotopic value of the atmosphere oxygen ($\delta^{18}\text{O} = 0$ ‰), while oxygen entering by diffusion will

Table 1. Summary of the stem chamber experiments. The D_{stem} error is calculated from the analytical uncertainty.

Tree	Site	Experiment month and year	Comments	Experiment duration (hours)	[O ₂] %	$\delta^{18}\text{O}$	D_{stem}	D_{stem} error
Aleppo Pine	HUJI	July 2010		141	19.67	0.06	15.2	0.5
Aleppo Pine	HUJI	December 2011		117	18.85	0.24	16.5	0.3
Aleppo Pine	HUJI	January 2011		48	19.30	-0.12	12.6	0.4
Aleppo Pine	HUJI	January 2011		48	19.30	-0.02	13.8	0.4
Stone Pine	HUJI	July 2010		141	18.56	0.21	16.0	0.3
Apple	HUJI	April 2010	after bud burst	51	17.89	1.06	21.4	0.3
Apple	HUJI	April 2010	after bud burst	51	17.80	1.11	21.5	0.3
Apple	HUJI	December 2010	green leaves	96	19.68	0.11	15.9	0.5
Apple	HUJI	December 2010	green leaves	117	19.68	0.09	15.5	0.5
Apple	HUJI	January 2011	green leaves	48	19.78	-0.06	13.0	0.5
Apple	HUJI	January 2011	green leaves	48	19.77	-0.12	12.0	0.5
Apple	HUJI	March 2011	no leaves	120	19.57	0.08	15.4	0.5
Apple	HUJI	March 2011	no leaves	144	19.44	0.03	14.5	0.4
Mari Mari 1	UNAP	April 2011	wet season	240	19.55	0.08	15.3	0.5
Mari Mari 3	UNAP	April 2011	wet season	240	18.83	0.11	15.2	0.3
Marupa 1	UNAP	April 2011	wet season	240	17.37	0.19	15.2	0.2
Marupa 2	UNAP	April 2011	wet season	240	16.76	1.08	19.5	0.2
Marupa 3	UNAP	April 2011	wet season	240	18.36	0.70	19.8	0.3
Tangarana 1	UNAP	April 2011	wet season	240	19.48	0.28	18.1	0.5
Tangarana 2	UNAP	April 2011	wet season	240	16.15	0.57	16.6	0.1
Tangarana 3	UNAP	April 2011	wet season	240	17.70	0.40	16.6	0.2

have negative $\delta^{18}\text{O}$ values (versus the atmosphere), due to fractionation by diffusion. Thus, if diffusion transport dominates, the oxygen in the chambers is expected to have negative $\delta^{18}\text{O}$ values that will increase with time as a result of back-diffusion (also with a discrimination of 14.1 ‰) and as a result of uptake by the stem. The results of this experiment, already published (Angert and Sherer, 2011), showed negative $\delta^{18}\text{O}$ values of -3.15 ‰ and -2.58 ‰, 2 and 4 h after the beginning of the experiment, respectively. The results of that experiment, as well as a fit of the model to it (achieved while assuming that the flushing with N₂ removed 90 % of the air in the chamber and stem pores next to it, and $D = 25$ ‰ which might be the result of the low O₂ stress) are shown in Fig. 2. Based on these two lines of evidence, and based on the absence of strong winds or strong diurnal temperature changes (especially in the tropical forest), we conclude that mass flow to the chambers was negligible. Another process that might introduce mass flow is changes in atmospheric pressures. However, we sampled during fair weather in which the atmospheric pressure changes (as recorded by nearby meteorological stations) were < 6 hPa. Our model shows that such pressure change will have an effect on estimate D in the order of 0.1 ‰. However, care should be taken to avoid setting such experiments during periods of sharp changes in pressure. The results of the blank experiment aimed to test the effect of water vapor diffusion (Severinghaus et al., 1996), are as predicted from theory – small and with negligible effect on our discrimination estimations.

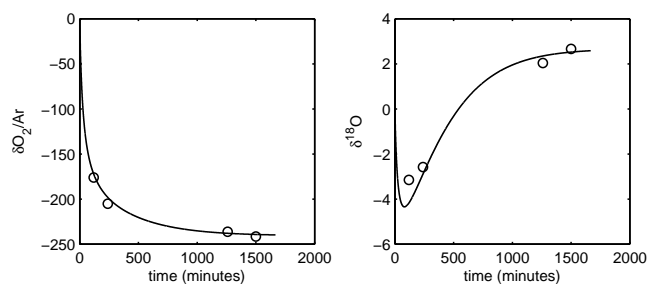


Fig. 2. Measured (open circles) and modelled (solid line) $\delta\text{O}_2/\text{Ar}$ values and $\delta^{18}\text{O}$ values (both in permil versus atmospheric air) during the experiment which started with flushing the chamber on the Apple tree with N₂. The model assumes constant respiration rate and discrimination. The results of this experiment are consistent with diffusion being the main driver of gas exchange in the chamber.

We found frequent occurrence of low discrimination values, in the range of 12–16 ‰ which are well below the known values for COX respiration in isolated plant mitochondria, which is ~ 20 ‰, (Ribas-Carbo et al., 1995). The low values are most probably not related to variation in the COX in different plant species, since discrimination of ~ 20 ‰ was also reported for the much more genetically remote marine eukaryotes and marine bacteria (Kiddon et al., 1993). Hence, the low value we measured can be explained only by the effect of diffusion limiting the internal O₂ concentration. As was found for soils, limiting diffusion of O₂ to the

consumption site makes the effective discrimination lower than the one involved in the enzymatic respiration process by itself with no limit to the supply of O₂. Again as in soils, we can suggest a conceptual model of “a box within box”, in which oxygen first diffuses in gas phase through the stem (“outer box”), and then diffuses in liquid phase to the consumption site within living cells (“inner box”). The discrimination by the combined effects of diffusion into the inner box and enzymatic O₂ consumption within it, is given by the following equation (after Farquhar et al., 1982):

$$D' = D_{\text{diff}} + (D_{\text{con}} - D_{\text{diff}})C_i/C_a, \quad (4)$$

where D' is the overall discrimination of the inner box, D_{con} is the discrimination in consumption, D_{diff} is the discrimination in diffusion to the inner box, and C_a and C_i are the O₂ concentrations outside and inside the inner box, respectively. The discrimination of the entire stem (D_{stem}) will relate to the discrimination of the inner box (D') by a similar equation:

$$D_{\text{stem}} = D_{\text{diff}} + (D' - D_{\text{diff}})C_i/C_a, \quad (5)$$

where D_{diff} is the discrimination in diffusion through the stem, which is 14.1 % assuming that this diffusion is in gas phase. According to Equations 4 and 5, the D_{stem} values below 14 % can only be explained if the diffusion to the inner box is by liquid phase diffusion, in which the discrimination is small (2.7 % discrimination was found for O₂ air-water gas transfer; Knox et al., 1992).

The differences observed between tree species could reflect variations in structural barriers to diffusion. Indeed, Marupa, the tropical tree in which the highest discrimination was measured, has a reported wood density of only 0.35 g ml⁻¹ versus 0.53 and 0.65 g ml⁻¹ for Mari-Mari and Tangarana (Chambers et al., 2004). However, the diffusivity depends not only on the density (and hence porosity) of the stem, but also on the water content which may vary, and on the structure of the air-filled pore spaces (Soriz and Hietz, 2006). We currently do not have data on diffusivity values of the live tissue.

Variations in diffusivity (resulting in lower discrimination values) cannot explain the discrimination measurements that are greater than those expected for the cytochrome (COX) pathway respiration (20 %). The only known process that can result in higher discrimination values is the engagement of the AOX respiration pathway. Moreover, since it is reasonable to assume that the diffusion limitation always exists, the discrimination values above 19.5 % as those in some of the Marupa trees, also indicate large contributions of the AOX pathway (relative to the COX), which shift the overall discrimination to higher values, and compensate for the effects of diffusion. With time, the changes in discrimination in the Apple tree resemble variation in the range of 12.3 to 19.9 %, observed for the same root system in the course of a few weeks (Angert and Luz, 2001), and are probably also related

to changes in the relative contribution of AOX to total respiration O₂ consumption.

Our findings have implications for estimating the average land contribution to the Dole Effect, as well as for estimating the variability and uncertainty of this value. The average of the stem experiments in the current study is 16 %. However, we observed large variability in discrimination values with season, between species, and within species, so this value cannot be assumed to be globally representative. If the value of globally averaged discrimination in stem uptake happens to be 16 %, then assuming that stem's respiration consists of 16 % (Litton et al., 2007) of the land respiration, the estimation of the land contribution to the Dole Effect will be lower than 0.3 % relative to an estimate that uses a discrimination value of 18 % for all land dark respiration (Blunier et al., 2002). This 0.3 % value is small in comparison to other uncertainties in estimating the land Dole Effect (Luz and Barkan, 2011). However, the paleo data point to variability in the land Dole Effect in the order of only 0.5 % (Severinghaus et al., 2009). If we assume a shift from an average stem discrimination of 15 % to 19 % (well within our measured range of 12.0–21.5 %), it will induce a shift of ~0.6 % in the land Dole Effect. This effect is in similar magnitude to that resulting from possible changes in the discrimination by soils, which were suggested (Severinghaus et al., 2009) to partly explain past changes in the Dole Effect. This soils effect was suggested to result from variations in the activity of tropical soil respiration, shown to be diffusion-limited and have low discrimination, relative to the activity of boreal forest, where engagement of AOX resulted in high discrimination. However, given that to date there are only two surveys of the discrimination in various soil sites (Angert et al., 2003, 2012), and considering the variability reported here for stem discrimination, many more studies must be conducted before the variability in the Dole Effect can be reliably interpreted.

Another important implication of our findings is for estimating the contribution of the AOX to respiration and the carbon cycle. To the best of our knowledge, this study is the only one to date that has estimated AOX activity in intact plants in the field. Given the known relation between AOX and various types of stress (Vanlerberghe and McIntosh, 1997; Moore et al., 2002; Rachmilevitch et al., 2007), working with intact plants has a clear advantage over incubating desiccated plant tissue (Ow et al., 2008; Searle and Turnbull, 2011) that might exhibit a wound response. Using the approach demonstrated here, it is possible to trace how the AOX contribution in woody tissue of a single plant changes seasonally, and due to environmental conditions such as temperature and soil moisture. For example, we found the highest discrimination in the Apple tree shortly after bud burst, which somewhat resembles the high discrimination reported for aquatic systems shortly after spring bloom (Luz and Barkan, 2011). These findings are in agreement with findings of increased AOX activity shortly after changes in environmental conditions, or before an increase in the rates of respiration through

the COX (Moore et al., 2002; Rachmilevitch et al., 2007). As noted above, the AOX is also reported to be activated as a response to stress. We thus believe that more field studies of the AOX will improve the understanding of the carbon cycle and its sensitivity to climate induced changes in respiration.

5 Conclusions

We have measured, for the first time, the O₂ discrimination associated with respiration of intact tree stems. We have found, as predicted from theory, evidence for both the effect of slow diffusion that limits the internal O₂ concentrations and lowers the discrimination, and the effect of the AOX that increases it. As a result of these two contrasting processes, the discrimination in stem uptake is highly variable and covers the range of 12.6–21.5 ‰. Our findings have implications for understanding past variations in the Dole Effect, and for tracing the AOX activity in intact plants at the field.

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