Running head (<50 characters):
Drought, photosynthesis and isoprenoid emission

Corresponding authors:
Kaidala Ganesha Srikanta Dani,
Brian James Atwell,
Department of Biological Sciences,
Macquarie University,
North Ryde,
Sydney, NSW 2109
Australia

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Macquarie University, North Ryde, Sydney, NSW 2109, Australia

Corresponding authors; email srikantadani@yahoo.co.uk; brian.atwell@mq.edu.au
Increased ratio of electron transport to net assimilation rate supports elevated isoprenoid emission rate in eucalypts under drought

Kaidala Ganesha Srikanta Dani*, Ian McLeod Jamie, Iain Colin Prentice, Brian James Atwell*

Department of Biological Sciences, Macquarie University, North Ryde, Sydney, NSW 2109, Australia (K.G.S.D., I.C.P., B.J.A.); Department of Chemistry and Biomolecular Sciences, Macquarie University, North Ryde, Sydney, NSW 2109, Australia (K.G.S.D., I.M.J.); and AXA Chair of Biosphere and Climate Impacts, Grantham Institute for Climate Change and Grand Challenges in Ecosystems and Environment, Department of Life Sciences, Imperial College London, Silwood Park Campus, Buckhurst Road, Ascot SL5 7PY, United Kingdom (I.C.P.)

*Corresponding authors; email srikantadani@yahoo.co.uk; brian.atwell@mq.edu.au

Summary (<200 characters):

Volatile isoprenoids emitted by plants have a significant influence on ozone pollution and global climate. We show how changes in photosynthesis cause increased isoprenoid emissions in plants under drought.
Abstract (<250 words):

Plants undergoing heat and low CO₂ stresses emit large amounts of volatile isoprenoids compared with those in stress-free conditions. One hypothesis posits that the balance between reducing power availability and its use in carbon assimilation determines constitutive isoprenoid emission rates in plants and potentially even their maximum emission capacity under brief periods of stress. To test this, we used abiotic stresses to manipulate the availability of reducing power. Specifically, we examined the effects of mild to severe drought on photosynthetic electron transport rate (ETR) and net carbon assimilation rates (NAR) and the relationship between estimated energy pools and constitutive volatile isoprenoid emission rates in two species of eucalypts: *Eucalyptus occidentalis* (drought-tolerant) and *Eucalyptus camaldulensis* (drought-sensitive). Isoprenoid emission rates were insensitive to mild drought and the rates increased when decline in NAR reached a certain species-specific threshold. ETR was sustained under drought and the ETR/NAR ratio increased, driving constitutive isoprenoid emission until severe drought caused carbon limitation of the MEP pathway. The estimated residual reducing power unused for carbon assimilation, based on the energetic status model, significantly correlated with constitutive isoprenoid emission rates across gradients of drought ($r^2 > 0.8$) and photorespiratory stress ($r^2 > 0.9$). Carbon availability could critically limit emission rates under severe drought and photorespiratory stresses. Under most instances of moderate abiotic stress-levels, increased isoprenoid emission rates compete with photorespiration for the residual reducing power not invested in carbon assimilation. A similar mechanism also explains the individual positive effects of low CO₂, heat and drought stress on isoprenoid emission.
Introduction (<1000 words):

The emission of volatile isoprenoids by plants (globally amounting to ~1000 TgC yr\(^{-1}\), of which isoprene constitutes ~500 TgC yr\(^{-1}\)) plays a significant role in tropospheric oxidation chemistry (The Royal Society Report- Fowler et al., 2008). Plant isoprenoid emission at the ecosystem scale is determined not only by intrinsic biochemical and physiological controls but also by the relative abundances of species, each with characteristic baseline emission capacities and each subject to modification by environmental conditions (e.g. Harrison et al., 2013). While the effects of most environmental factors on isoprenoid emission have been documented (Loreto and Schnitzler, 2010), their interactions are likely to be complex and hold the key to accurate projections of global emissions (Arneth et al., 2007; Squire et al., 2014). The effect of soil water availability on plant volatile isoprenoid emission is crucial to the projections, especially as rainfall patterns are themselves subject to the impacts of climate change.

Volatile isoprenoid emission is notably insensitive to moderate drought (when fraction of available soil water ranges from 40 to 70%; Fortunati et al., 2008; Centritto et al., 2011). Given the various other consequences of drought for plant function viz., stomatal closure leading to reduced photosynthesis (Lawlor & Cornic, 2002); increased leaf temperature (Jones 2004); leaf shedding (Tyree et al., 1993); reduced growth and potential hydraulic failure (Maherali et al., 2004); reduced shoot-to-root ratio (Poorter et al., 2012); increased oxidative stress due to the activation of reactive oxygen species (Mittler & Zilinskas, 1994); and an increased sucrose-to-starch ratio, affecting osmotic adjustment (Chaves, 1991), it is not surprising that there are large variations in experimental and field measurements of isoprenoid emission in response to drought (reviewed in Laoothawornkitkul et al., 2009; Niinemets, 2010).

Isoprene emission involves an energy-intensive biosynthesis through the methylerythritol phosphate (MEP) pathway in chloroplasts (reviewed in Sharkey & Yeh, 2001). Photosynthesis contributes the required carbon skeletons and reducing power for isoprenoid biosynthesis, at least under stress-free conditions (reviewed in Loreto & Schnitzler, 2010). The photosynthetic energy and reducing power output of a chloroplast is shared in unequal proportions among co-localized pathways (Table 1). Under favourable conditions photosynthetic carbon reduction (PCR) is the largest energy sink (~50%). Abiotic stresses enhance the supply of energy and reducing power to non-PCR sinks in the chloroplast (Haupt-Herting & Fock, 2002). Examples include (a) increased photorespiration under drought, at least until Rubisco is directly affected by stress (e.g. Lawlor, 1976; Noctor et al., 2002);
(b) increased photorespiration under low-CO$_2$, low-light or high-light stress (Kozaki & Takeba 1996) and (c) increased photo-reduction of oxygen under photo-oxidative stress (Makino et al., 2002). It has been posited that isoprenoid emission is a mechanism to consume surplus reducing power in stressful high-light and/or low-CO$_2$ environments (Niinemets et al., 1999; Way et al., 2011). Increased accumulation of secondary metabolites such as phenols, alkaloids and isoprenoids have been documented in plants under abiotic stresses (reviewed in Wilhelm and Selmar, 2011). Decreased carboxylation and increased oxidative stress due to oversupply of reducing equivalents is seen as the main driver of increased secondary metabolism under drought (Selmar and Kleinwächter, 2013). Post illumination behaviour of isoprene emission (primary and secondary bursts) under O$_2$-free, pure N$_2$ atmospheres have been attributed to availability of reducing equivalents (Rasulov et al., 2011; Li & Sharkey, 2013). It has further been proposed that the MEP pathway competes with other sinks for reducing power, so that the flow of reducing power to isoprenoid biosynthesis is proportional to the energy unused for primary metabolism (Morfopoulos et al., 2013, 2014; Dani et al., 2014). However, the MEP pathway’s requirement for reducing power is very small relative to that of photorespiration (Sharkey et al., 2008) and given the diversity in the relative sink strengths of intra-plastidic processes (Table 1), it is still unclear how the demands of these different processes influence one another.

Eucalypts have been used as model systems to study plant isoprenoid emission since the early years of isoprenoid research (e.g. Guenther et al., 1991; Brilli et al., 2013). All eucalypts store monoterpenes as well as constitutively emit isoprene and some monoterpenes (e.g. He et al., 2000). *Eucalyptus camaldulensis* subsp. *camaldulensis* (River Red Gum) is a drought-avoiding mesic species that is tolerant to waterlogging and distributed in riparian, temperate south-eastern Australia (Farrell et al., 1996). *E. occidentalis* (Swamp Yate) is a drought-tolerant species found in saline environments in mediterranean south-western Australia (Benyon et al., 1999; Searson et al., 2004). *E. camaldulensis* subsp. *obtusa* is the most widespread eucalyptus in subtropical Australia (Butcher et al., 2009). Exploiting this ecological contrast, we empirically tested the hypothesis that the isoprene emission is driven by ATP and NADPH availability (Loreto and Sharkey, 1993; Niinemets, 1999) and could potentially compete for the same with carbon assimilation (Harrison et al., 2013; Morfopoulos et al., 2014). We manipulated the energy source-sink dynamics by imposing various abiotic stresses including drought, heat, low CO$_2$, and high O$_2$. We investigated the relationship between three plastidic biochemical processes (carbon assimilation, photorespiration and volatile isoprenoid emission) in eucalypts acclimated to drought for four to six months. Interactive effects of...
short-term exposure to five CO₂ concentrations, three O₂ levels and heat stress on isoprenoid emission rates were also analysed. It was hypothesised that the relative sink strength of various processes requiring reducing power in the chloroplasts could determine the variations of isoprenoid emission in plants experiencing abiotic stress. We started with a premise that the light-dependent and light-independent reaction components of photosynthesis have different susceptibilities to abiotic stress (particularly to drought) and that these susceptibilities vary across species.

Results:
The results are from three independent experiments (see methods, Table 2).

Photosynthesis
(a) Acclimation to drought in paired species (at 20% O₂): *E. occidentalis* had a significantly higher stomatal conductance ($g_s$, $P = 0.043$) when watered to field capacity (FC) than *E. camaldulensis* subsp. *camaldulensis* but the difference in transpiration rates was not significant ($T_r$, $P = 0.193$). Net assimilation rate (NAR) of both *E. occidentalis* (16.8 ± 2.28 µmol m⁻² s⁻¹) and *E. camaldulensis* subsp. *camaldulensis* (18.1 ± 1.86 µmol m⁻² s⁻¹) were comparable (test of equal means, $P = 0.001$). During acclimation to severe drought stress (FC ≤ 50%), *E. camaldulensis* subsp. *camaldulensis* showed a significant decline in all photosynthetic parameters ($P < 0.001$) whereas net assimilation in *E. occidentalis* (15.3 ± 1.81 µmol m⁻² s⁻¹) although decreased, remained comparable to control values despite a significant decrease in stomatal conductance (Fig. 1A, 1D). Under well-watered conditions, estimates of photosynthetic linear electron transport rate (ETR) based on chlorophyll fluorescence for *E. occidentalis* and *E. camaldulensis* subsp. *camaldulensis* showed a consistent proportionality with their respective NARs. For *E. occidentalis* the ETR/NAR ratio remained unchanged even at 50% FC while the ratio significantly increased (doubled) for *E. camaldulensis* (Fig. 2A). The carbon cost of isoprenoid emission as a proportion of net assimilation increased >10 fold as drought intensified and was highest in both species at 25% FC (Fig. 2B). ETR was measured independently using fluorescence and was not coupled with LiCor measurements (Experiment 1, Table 2). Hence, the observations should be treated only as indicative and not as absolute. ETR/NAR ratios (~7:1 under 20% O₂) were more realistic when obtained after fitting $A$-$C_i$ curves (Experiment 3; Fig. S3B). ETR/NAR ratio across drought treatments follow a near-significant quadratic regression with total emission rates ($r^2 = 0.75$; $P = 0.12$; Fig. 2C).
(b) **Response to heat and CO<sub>2</sub> in *E. camaldulensis* subsp. obtusa**: Heat caused a significant decline in photosynthesis under elevated CO<sub>2</sub> (≥1000 µmol mol<sup>−1</sup>, *P* < 0.001). Leaves at 38°C transpired at a significantly higher rate than those at 28 °C (*P* < 0.0001). Heat did not cause a significant change in either *g*<sub>s</sub> or *T*<sub>r</sub> under normal O<sub>2</sub> in plants under drought (50% FC). This was true across most of the CO<sub>2</sub> range (400 to 1800 µmol mol<sup>−1</sup>), except at 180 µmol mol<sup>−1</sup> CO<sub>2</sub> and at the photorespiratory compensation point (60 µmol mol<sup>−1</sup>, normal O<sub>2</sub>). Leaves of well-watered plants did not show a decline in NAR when subjected to 38 ºC under present-day ambient CO<sub>2</sub> (400 µmol mol<sup>−1</sup>) and normal O<sub>2</sub> (Fig. 3A). Heat had a generally positive effect on *T*<sub>r</sub>, under normal O<sub>2</sub> at 100% FC (but not under drought) and it was pronounced at CO<sub>2</sub> ≤ 400 µmol mol<sup>−1</sup> (Fig. S1).

(c) **Response to varying O<sub>2</sub> concentration**: Net assimilation rate in both species significantly increased (>30%) during low O<sub>2</sub>. The gain was proportional to their respective basal rates under normal O<sub>2</sub> except for *E. camaldulensis* subsp. *camaldulensis* at FC ≤ 50% (Fig. 1B, S3). During low O<sub>2</sub>, *T*<sub>r</sub> did not change significantly at 100% FC (equal means, *P* = 0.002) despite decreases in *g*<sub>s</sub> and (as a result) a decrease in leaf-internal CO<sub>2</sub> (*C*<sub>i</sub>). *T*<sub>r</sub> was insensitive to CO<sub>2</sub> concentrations under low O<sub>2</sub> in *E. camaldulensis* subsp. obtusa (Fig. S1, *P* > 0.1).

Low O<sub>2</sub> had a significant negative effect on transpiration and net assimilation in *E. camaldulensis* subsp. *camaldulensis* only under acute water deficit (Fig. 1C, FC ≤ 50%, *P* < 0.0001, also see Fig. S3A). High O<sub>2</sub> (50% O<sub>2</sub>) caused significant increase in *C*<sub>i</sub>, severe decline in net assimilation rate in *E. camaldulensis* subsp. *camaldulensis* and the effect was persistent and amplified under drought (Table S1).

**Volatile isoprenoid emission**:

(a) **Response to drought**: Branch-level basal isoprene emission rate (*I*<sub>e</sub>) at 25 °C, 1200 µmol m<sup>−2</sup> s<sup>−1</sup> PAR, and present day CO<sub>2</sub> and normal O<sub>2</sub> was 40% higher (*P* = 0.004) in *E. camaldulensis* subsp. *camaldulensis* (5.9 ± 1.48 nmol m<sup>−2</sup> s<sup>−1</sup>) than in *E. occidentalis* (3.4 ± 1.99 nmol m<sup>−2</sup> s<sup>−1</sup>) and the rates remained unchanged in both species despite acclimation to moderate drought (70% FC). The trends were conserved in leaf level measurements. There was a tiny decrease (relative to control) in net assimilation rate in *E. occidentalis* at 50% FC (∆NAR= −1.5 µmol m<sup>−2</sup> s<sup>−1</sup>) and it was accompanied by a marginally significant increase in isoprene emission rate (∆*I*<sub>e</sub> = +1.4 nmol m<sup>−2</sup> s<sup>−1</sup>; Fig. 1E; *P*=0.05; Note: There is three orders of magnitude difference between amounts of carbon fixed via photosynthesis and carbon lost via emission). The biggest increase in *I*<sub>e</sub> for these two species occurred at two different drought intensities. *I*<sub>e</sub>
peaked significantly at 50% FC for *E. camaldulensis* (*P* < 0.005, Fig. 1E), and at 25% FC for *E. occidentalis* (*P* < 0.001). These emission peaks coincided with the first noticeable increase in their respective ETR/NAR ratios (Fig. 2A, 2C) but the emission declined for *E. camaldulensis* subsp. *camaldulensis* although ETR/NAR increased further at 25% FC. Constitutive monoterpane emission rate, *M*<sub>e</sub> (pinenes and d-limonene) in *E. camaldulensis* subsp. *camaldulensis* behaved in a manner similar to isoprene while *M*<sub>e</sub> in *E. occidentalis* did not respond to drought even at 25% FC. *I*<sub>e</sub> and *M*<sub>e</sub> in both *E. occidentalis* and *E. camaldulensis* subsp. *camaldulensis* were comparable in magnitude across the drought gradient (Fig. 1E and 1F). *I*<sub>e</sub> to *M*<sub>e</sub> molar ratio was roughly 10:1 in both species. The subspecies *obtusa* showed a basal *I*<sub>e</sub>*/M*<sub>e</sub> molar ratio of ~2:1.

(b) **Response to heat and CO<sub>2</sub> in *E. camaldulensis* subsp. *obtusa***: Heat was the most significant factor causing an increase in *I*<sub>e</sub> (*P* < 0.0001) and *M*<sub>e</sub> (*P* < 0.001) across all treatments. At 28 °C, 100% FC (*N* = 6) and 20% O<sub>2</sub>, *I*<sub>e</sub> showed a significant peak at 180 µmol mol<sup>-1</sup> CO<sub>2</sub> (*P* < 0.001) and almost full inhibition at the saturating CO<sub>2</sub> level of 1800 µmol mol<sup>-1</sup> (Fig 3B and 4). This response completely disappeared at 38 °C (Fig. 3B). Under normal O<sub>2</sub>, the *I*<sub>e</sub> response at 50% FC without heat stress (28 °C) was equivalent in magnitude to *I*<sub>e</sub> observed in the well-watered plants subjected to heat stress (38 °C; *P* < 0.001) irrespective of CO<sub>2</sub> acclimation (Fig. S5). Plants acclimated to drought (50% FC) and exposed to heat stress (38 °C) showed the highest isoprenoid emission. High-CO<sub>2</sub> induced inhibition of isoprene emission at 28 °C disappeared at 38 °C and was accompanied by a significantly low stomatal conductance and low leaf internal CO<sub>2</sub> (Fig. S1).

(c) **Response to varying O<sub>2</sub> concentration**: Exposure to 2% O<sub>2</sub> (10 min) resulted in a marginal increase in *I*<sub>e</sub> and *M*<sub>e</sub> across the drought gradient (Fig. 1E and 1F) and these trends were conserved in both *E. occidentalis* and *E. camaldulensis* subsp. *camaldulensis*, except that the latter showed no significant change in *I*<sub>e</sub> at 100% and 70% FC. Low O<sub>2</sub> on its own did not significantly affect *M*<sub>e</sub> in *E. occidentalis* (Fig. 1f) or in *E. camaldulensis* subsp. *obtusa* (*P* > 0.6, Fig. 3C). Low O<sub>2</sub> significantly increased *I*<sub>e</sub> at CO<sub>2</sub> = 1800 µmol mol<sup>-1</sup> (no heat stress) compared to lower CO<sub>2</sub> levels, which was opposite to the effect under normal O<sub>2</sub> (*P* < 0.0001). Low O<sub>2</sub> also had a positive effect on the *I*<sub>e</sub> of well-watered plants at 38 °C. In *E. camaldulensis* subsp. *camaldulensis* *I*<sub>e</sub> increased when well-watered plants were exposed to 50% O<sub>2</sub> (relative to plants at 20% O<sub>2</sub>). Emission rate decreased significantly when the plants simultaneously experienced drought (50% FC) and high oxygen (50% O<sub>2</sub>). The effect of low
O₂ on \( I_e \) was more pronounced than on \( M_e \) in all three taxa (\( P < 0.001 \)) and increased \( I_e \) under low O₂ resulted in a decreased \( M_e \) in well-watered plants (Fig. 5A, 5C).

The relationship between leaf energetic status and isoprenoid emission rate: The estimated electron transport rate not used for light independent reactions (\( J_l \)) correlated significantly and positively with isoprenoid emission rate in both species (\( r^2 = 0.81; P = 0.014 \)) and it was consistent across drought gradient (Fig. 4A). The positive relationship between \( J_l/V_{cmax} \) (derived from \( A-C_i \) curves) and isoprenoid emission rate (Fig. 4B) was consistent with the ETR/NAR ratio peaking with increased emission in both species. The relationship between \( J_l \) and \( I_e \) estimated at three different oxygen concentrations was significantly positive (\( r^2 > 0.91; P = 0.02 \)) for \( E. \) \textit{camaldulensis} subsp. \textit{camaldulensis} (Fig. 5A). Plants experiencing drought (50% FC) exposed to 50% O₂ were the only exception to the trend and showed a significant decline in isoprenoid emission despite large \( J_l \) (Fig. 5A). \( E. \) \textit{camaldulensis} subsp. \textit{camaldulensis} exposed to extreme photorespiratory stress without drought (100% FC, 50% O₂) and drought without extreme photorespiration (50% FC, 20% O₂) resulted in large increases in \( J_l/V_{cmax} \) (Fig. 5B).

Discussion:

Drought, ETR/NAR ratio and constitutive isoprenoid emission

The insensitivity of ETR to drought in both species in this study confirmed that the photosystems and the electron transport chain are not susceptible to moderate drought stress (Ben et al., 1987) and the relative decrease in ETR under drought is proportionately less when compared to decrease in CO₂ assimilation (Cornic and Briantais, 1991, Bota et al., 2004). The absolute rates of isoprene and constitutive monoterpane emission did not change provided that the simultaneous assimilation rate of CO₂ remained unchanged.

The mediterranean species \textit{Eucalyptus occidentalis} maintained almost an unchanged NAR and isoprene emission rate even at 50% FC (Fig. 1D and 1E). \( E. \) \textit{camaldulensis} subsp. \textit{camaldulensis} showed a gradual and more marked decline in NAR with increasing water deficit. ETR/NAR ratio significantly increased at 25% FC for the former and at 50% FC for the latter, the point where the species showed its highest isoprene and monoterpane emission rates (Fig. 1, 2A, 2C). We attribute the increased isoprene emission rates under drought to increased ETR/NAR ratio and increased availability of reducing power to the MEP pathway among other non-PCR sinks (Fig. 4). Under severe drought (25% FC) despite a favourable ETR status of its leaves, isoprenoid emissions of \( E. \)
Eucalyptus camaldulensis subsp. camaldulensis declined suggesting carbon limitation despite 2% oxygen (see decline in NAR at %FC ≤50; Fig. S3A). It was later confirmed that low O\textsubscript{2} exposure did not significantly affect ETR/NAR ratios both under well-watered and droughted conditions (Fig. S3B). Similarly, imposing severe photorespiratory stress (50% O\textsubscript{2}) on well-watered plants resulted in increased isoprene emission rate and the rates plummeted under drought despite a large pool of residual reducing power (Fig. 5). The results suggested that non-PCR reactions (especially photorespiration and the MEP pathway) compete for reducing power not allocated to carbon assimilation reactions under situations of sub-optimal carbon assimilation due to abiotic stress. Although eucalypts may only have a modest capacity for non-photochemical quenching (NPQ) due to their typically high photosynthetic capacities and acclimation to high-light habitats, the proportion of energy dissipated through NPQ, which is one of the primary mechanisms to mitigate oxidative stress, increased in *E. camaldulensis* subsp. *camaldulensis* as drought intensified (Fig. S2). NPQ is likely to be a significant sink for ETR and may also account for reduced emissions under severe stress (Fig. 5A).

### Drought, photorespiration and constitutive isoprenoid emission

The direction of change in the rates of isoprene emission and photorespiration in response to many environmental factors are same, despite absence of a biochemical (carbon based) link between the two pathways (e.g. Monson and Fall, 1989; Hewitt et al., 1990; Loreto and Sharkey, 1990). In this study, net assimilation and isoprene emission rates increased by a small yet significant extent in well-watered *E. occidentalis* exposed to short-term low O\textsubscript{2} (Fig. 1D, 1E; in agreement with Hewitt et al. 1990) and such an increase persisted under drought. Increased *de novo* carbon pool and decreased competition for ATP and NADPH may explain the small increase in isoprene emission under low O\textsubscript{2} in well-watered plants, given that ATP could be limiting emissions when carbon is plentiful (Loreto and Sharkey, 1993). Although increased isoprene emission in low O\textsubscript{2} under most conditions may be physiologically important, it is not comparable to the large difference in emission between well-watered plants (low emission) and plants experiencing drought (high emission) under 20% O\textsubscript{2} (Fig. 1E). Increased emission under drought is sustained so long as the intensity of drought is within a species-specific tolerance threshold. When such a threshold is exceeded, isoprene emission rate significantly decreased (at 25% FC for *E. camaldulensis* subsp. *camaldulensis*; Fig. 1E). Since we did not directly estimate photorespiration rates under drought (which is known to significantly increase under abiotic stress), we increased photorespiratory stress in well-watered plants to mimic alternative scenarios. When photorespiratory stress is extreme (50% O\textsubscript{2}) and it is coupled with
reduced carbon assimilation capacity due to drought (50% FC), the MEP pathway suffers a ‘double
jeopardy’ and is likely deprived of both carbon and reducing power (Fig. 5A).

The cost of carbon due to isoprenoid emission increased nearly 15 fold from 0.46% of freshly fixed
carbon in well-watered plants to 7.2% in severely stressed *E. camaldulensis* subsp. *camaldulensis*
(Fig. 2B). Although alternative carbon imported from the cytosol avoids carbon-limitation of the
MEP pathway under moderate abiotic stress (Brilli et al., 2007; Funk et al. 2004; Trowbridge et al.,
2012), under extreme stress carbon could be limiting due to irreparable biochemical impairment of
both the PCR cycle and rates of carbon import into plastids. Whether drought just inhibits carbon
assimilation rate through stomatal diffusional limitation or there is clear biochemical down-
regulation is highly debatable (e.g. Flexas et al., 2004). Under severe drought stress (especially for *E.
camaldulensis* subsp. *camaldulensis*) even those limited number of leaves that were retained by the
plants could not have recovered fully if the plants were re-watered. In such cases, diffusional
limitation was likely compounded by impairment of photosynthetic biochemical machinery. Limited
catalytic activity of the MEP pathway, particularly isoprene synthase (Brilli et al., 2007), could have
contributed to decreased emissions under extreme stress.

**Drought, low CO₂, heat and constitutive isoprenoid emission**

In *E. camaldulensis* subsp. *obtusa*, short-term acclimation to heat stress (38 °C) caused significantly
higher emissions with no significant change in net assimilation despite drought (50% FC, Fig. 3).
CO₂ inhibition of isoprene emission also disappeared at high temperatures (as reviewed in Sharkey
and Monson, 2014). It is known that moderate heat stress can suppress ETR yet increase both *V*ₘₐₓ
and isoprene emission rate (e.g. Dreyer et al., 2001; Darbah et al., 2008). Cold and heat treatments
are shown to selectively suppress PS II (linear electron transport) and thus reduce NADPH
availability and up-regulate PS I (cyclic electron transport) to increase ATP production (e.g. Huner et
al., 1993; Zhang and Sharkey 2009). All of these observations appear to contradict the view that
reducing power availability (however small may the requirement be) influences variation in volatile
isoprenoid emission. For the moment if we ignore cold stress, which is not relevant to isoprene
emission, at least to the extent we understand the phenomenon today, the energetic status model may
be inadequate to explain emission behaviour at high temperatures for the following reasons (a)
prolonged heat stress reduces net assimilation rate (despite an increase in *V*ₘₐₓ), primarily due to
decreased CO₂ solubility and decreased Rubisco–CO₂ affinity (Sage and Kubien, 2007); (b)
prolonged heat and drought stress (when imposed together) reduce emission, possibly due to heat
sensitivity of the cytosolic carbon pool (Fortunati et al., 2008; Centritto et al., 2011); and (c) extreme stress not only reduces net assimilation rates but also increases photorespiratory drain on carbon and reducing power (Fig. 5).

Unlike isoprene emission, the response of constitutive monoterpene emission did not follow a consistent pattern across any of the treatments (Fig. 1F and 3C). In *E. camaldulensis* subsp. *camaldulensis*, constitutively emitted monoterpenes behaved like isoprene while in *E. occidentalis*, monoterpene emission was not sensitive even to severe drought. This could be partly due to sustained monoterpene synthase activity during drought, as reported in evergreen oaks (Lavoir et al., 2009). Oddly, isoprene emission increased in leaves simultaneously exposed to 28 °C, 1800 µmol mol\(^{-1}\) CO\(_2\) (not saturating for eucalypts) and 2% O\(_2\) (Fig. 3B). For reasons unknown, the same leaves also showed a significant (16%) decrease in net assimilation rate (Fig. 3A). We speculate that the possible (temporary) inhibition/down-regulation of other non-PCR sinks of reducing power such as photoassimilation of nitrogen under very high CO\(_2\) (Bloom et al., 2002) could have also contributed to increased isoprene emission. However, it is acknowledged that the PCR cycle and nitrate assimilation, both do not directly compete for reducing power (Robinson, 1988). A trade-off between isoprene and monoterpene emissions in response to CO\(_2\) (28 °C; Fig. S4) and low O\(_2\) (Fig. 5C) indicates that emission of isoprene and monoterpenes is inversely related (also see Harrison et al., 2013).

Increased secondary metabolism and specifically increased isoprene emission under drought could be involved in protecting photosystems against transient periods of oxidative and heat stress, as seen in some transgenic studies, although the mechanisms are unclear (Behnke et al., 2007; Velikova et al., 2011; Selmar and Kleinwächter, 2013; Ryan et al., 2014). However, the tiny increase in isoprene emission when photorespiration (the largest photoprotective sink) is suppressed, despite down-regulation of PCR under drought, suggests that the MEP pathway has limited capacity to oxidize the pool of excess reductants available under abiotic stress. The increased isoprenoid emission rates among plants experiencing drought (without heat stress), heat (without drought stress; Fig. S5) and artificially increased photorespiratory stress (without drought and without heat at 50% O\(_2\)) were quantitatively equivalent and more experiments are needed to differentiate the underlying mechanisms between these responses.

**Conclusions:**
The energy (ATP) and reducing power (NADPH) budget of the chloroplast are used in a hierarchical fashion. The PCR cycle dominates while, possibly, all other reducing sequences co-localized in the chloroplasts must compete for the remaining pool (Table 1). The equilibrium between the source (light reactions) and major sinks (carbon reduction and photorespiration) of energy, as well as sources (de novo and stored) and sinks (all anabolic processes) of carbon, becomes distorted under drought stress. Drought-induced reduction in the PCR cycle is accompanied by an increase in ETR/NAR ratio and a significant increase in volatile isoprenoid emission. The qualitative response of isoprenoid emission under drought may be similar among species, but the degree of drought-induced shift in the \( J/V_{\text{cmax}} \) ratio is species-specific. While energy-availability is clearly the common factor that underpins the individual effects of low \( \text{CO}_2 \) (Morfopoulos et al., 2014), heat, drought and photorespiratory stress (this study) on isoprenoid emission, the complex interactive effects of heat, \( \text{CO}_2 \) and drought seem to defy simple assumptions and remain largely uncertain (Fig. 3). Variation in atmospheric \( \text{O}_2 \) concentration between 10 to 35% during the last 200 million years (Falkowski et al., 2005) and its influence on carboxylation efficiency could have also played an important role in regulating global isoprene emissions on a macroevolutionary time scale. All of these indicate a need for a wider experimental analysis across different functional plant types and ecosystems if we are to reliably ‘scale up’ volatile isoprenoid emissions from plants to large regions.

Materials and methods:
The study included three independent and yet mutually supporting experiments (Table 2).

Rationale for selecting species: A group of 15 species of eucalypts belonging to distinct biomes within Australia were screened for photosynthetic performance and isoprenoid emission potential in March 2012. The first experiment involved a paired comparison of isoprenoid emission rates and photosynthesis in *Eucalyptus occidentalis* and *Eucalyptus camaldulensis* subsp. *camaldulensis* in response to drought acclimation. *E. camaldulensis* subsp. *camaldulensis* and *E. occidentalis* were studied as a pair in Experiment 1, as both had comparable photosynthetic capacities and both predominantly emitted isoprene and some monoterpenes at significant levels. A desirable contrast in the physiology of their water-relations is already highlighted (see introduction).

The second experiment tested whether known \( \text{CO}_2 \) and heat responses of isoprenoid emission are consistent under drought acclimation. The idea was to test any potential pathway discrimination towards either isoprene or monoterpene emission under abiotic stresses. *E. camaldulensis* subsp. *obtusa* was selected for Experiment 2, because it emitted comparable quantities of isoprene and constitutive monoterpenes.
The third experiment was a supplementary exercise that was inspired by the results of the first experiment. The third experiment explicitly tested the relationship between photorespiration and isoprenoid emission under drought in *E. camaldulensis* subsp. *camaldulensis*.

Eucalypts store monoterpenes and it is difficult to estimate instantaneous carbon and energy invested in monoterpenoid biosynthesis. However, we took necessary precautions to rule-out monoterpane emissions from stored pools (from leaf glands). Even if one considers both constitutive and stored monoterpenes together, the quantities are smaller (in the paired species of experiment 1) than that of isoprene emission by an order of magnitude. Besides, it is recently shown that stored monoterpenes are quantitatively insensitive to drought stress in eucalypts although there are clear qualitative variations and inconsistent trends in secondary metabolite accumulation (phenolics and terpenoids) in various plants under drought (Brilli et al., 2013; Selmar and Kleinwächter, 2013).

**Plant Material:** Seeds of *Eucalyptus camaldulensis* subsp. *camaldulensis* (Dehnh), *E. occidentalis* (Endl) were obtained from the Australian Tree Seed Centre at CSIRO (Canberra) and germinated in May 2012. Two-to-three-month-old seedlings (*N* = 8 per species) were transplanted to large pots comprising ~80 kg of red soil (from the Robertson area in NSW) and the required quantities of Osmocote® slow release fertilizer. An independent group of *E. camaldulensis* subsp. *obtusa* (Blakely) (*N* = 6) and was established in similar large pots. The plants were grown under the open sun with regular watering. Six-month-old saplings (Dec 2012) were transferred to and kept until the end of the experiment in a glasshouse maintained at a 25 °C/18 °C diurnal temperature cycle and natural photoperiodic regime. A third independent group of *Eucalyptus camaldulensis* subsp. *camaldulensis* (*N* = 5) were germinated in March 2013 and grown in a similar manner for one year (used for experiment 3). These plants were maintained at 100% FC and isoprenoid emission rates were determined at three different oxygen levels (2, 20 and 50%) in April-May 2014. After the measurements were complete, the plants were droughted to achieve 50% FC and maintained (over 10 days). Gas exchange measurements and volatile sampling were repeated.

**Water relations:** The soil water holding capacity was determined by water saturation and weighing. Five-month old saplings were watered and weighed (pot + plant) to obtain the 100% field capacity (FC) reference point. Plants were grouped into two sets of four biological replicates per species. One set was maintained at 100% FC throughout the experiment while the other received reduced water to achieve 70% FC (within two weeks) and maintained thereafter for three months. After volatiles were sampled from plants acclimated to 70% FC, watering was further reduced to achieve
50% FC and acclimated for two weeks followed by volatile sampling (repeated for 25% FC). One batch of *E. camaldulensis* subsp. *obtusa* was acclimated to 50% FC for one month before volatile sampling. The difference in acclimation period between experiment 2 and 3 is due to the species involved. In experiment 2 we had *E. camaldulensis* subsp. *obtusa*, which comes from lower latitudes of Australia and it grows in some of the driest places on the continent. It could endure longer periods of drought and also took longer to acclimate (checked by measuring stomatal conductance on randomly selected leaves) while *E. camaldulensis* subsp. *camaldulensis* stabilised more quickly as well as reflecting the effects of drought sooner. In experiment 3 we had only *E. camaldulensis* and we could shorten the acclimation period. The duration of severe stress was shorter than the duration at 70% FC to avoid severe defoliation (especially in *E. camaldulensis* subsp. *camaldulensis*) that would have otherwise hampered emission measurements. Leaf water potential was determined (experiment 1) using a 12-channel thermocouple psychrometer (JRD Merril Specialty Equipment, Logan, Utah, USA) calibrated at 25 °C using standard sodium chloride solutions (Lang 1967). Leaf discs (diameter = 0.5 cm) were bored out at predawn from one half of a fully expanded leaf and transferred and sealed in the psychrometer (10 leaves per treatment group). The chambers were equilibrated in a water bath at 25 °C for 3 hr before signal acquisition. The procedure was repeated on the following midday using a leaf disc punched out from the other half of the same leaf. Measurements were repeated at three time points during drought treatment (see Fig. 6 for the relationship between leaf water potential and % field capacity for a physiological assessment of drought intensity).

**Monitoring photosynthesis under drought:**

(a) Photosynthetic gas-exchange measurements were made using a LI-6400XT (Li-Cor Biosciences, Lincoln, Nebraska, USA) infrared gas analyser. During branch-level sampling, photosynthesis was measured between 12 noon and 3 pm in parallel to isoprenoid sampling on independent branches of the same plant. Leaf temperature was 25 °C, light intensity was 1200 μmol m⁻² s⁻¹, and relative humidity ranged from 39 to 47%. *A*-*C* _i_ curves were obtained from one or two of the best performing healthiest leaves from each biological replicate (*N*=4) per treatment group using a Li-Cor6400 at 25 °C and normal O₂ (also at 2% and 50% O₂ for experiment 3). *V* _cmax_ and *J* were estimated using the curve-fitting tool described in Sharkey et al., 2007 (minimised errors) and the mean values were used as model input parameters. *J* _r_ (the portion of the electron transport rate not used for dark
reactions of photosynthesis, given $C_i$ and $V_{cmax}$ was calculated following Harrison et al., (2013) and Morfopoulos et al., (2014).

$$J_v = 4V_{cmax} \left[ \frac{C_i + 2\Gamma^*}{C_i + K_m} \right]$$ (1)

$$J_r = (J - J_v)$$ (2)

Given $V_{cmax}$, we calculated $J_v$ and then substituted (1) in (2) where, $J_v$ = proportion of electron transport used for dark reactions; $V_{cmax}$ = maximum carboxylation rate by Rubisco; $K_m$ = effective Michaelis-Menten coefficient for carboxylation by Rubisco (700 µmol mol$^{-1}$ at 25 °C); $\Gamma^*$ = photorespiratory compensation point (60 µmol mol$^{-1}$); $C_i$ = species-specific leaf internal CO$_2$ concentration (µmol mol$^{-1}$) at ambient CO$_2$ = 400 µmol mol$^{-1}$.

(b) Chlorophyll fluorescence was monitored using a Pocket PAM chlorophyll fluorescence meter (Gademann Instruments GmbH, Wurzburg, Germany). Kautsky dark-light fluorescence induction curves were obtained from dark adapted leaves (pre-dawn) between 3 and 5 am and replicated on 15 fully expanded leaves per treatment group ($N=4$, biological replicates). The measurements were made in a dark room (PPFD < 10 µmol m$^{-2}$ s$^{-1}$). Pre-dawn leaf temperature was 15.5 ± 0.3 °C. During the day (between 2 and 4 pm), leaves experiencing moderate light levels (350 < PPFD < 450 µmol m$^{-2}$ s$^{-1}$) were used to estimate linear electron transport rates using steady-state light induction curves by gradually increasing the pulse intensity from 0 to 1500 µmol m$^{-2}$ s$^{-1}$. Pre-dusk leaf temperature was 24.5 ± 0.8 °C. The data for ETR/NAR was measured only after the dry-group had been acclimated to 50% FC and the control group (100% FC plants) was retained through-out the experiment.

Volatile isoprenoid sampling

(a) Branch enclosure method: 25 L capacity Tedlar® bags (Sigma) were modified to make a volatile collection chamber with a PTFE (polytetrafluoroethylene) base for air tight sealing. The gas exchange line was plumbed with Teflon tubing and stainless steel air tight connectors (Swagelok®). High-purity instrument-grade air (BOC; 78% N$_2$, 21% O$_2$, 1% Ar) was mixed with CO$_2$ (beta mix 5% ± 0.1% in N$_2$) to achieve ambient CO$_2$ (400 ± 10 µmol mol$^{-1}$) concentration in the head space containing the branch. The unit was flushed at 20 L min$^{-1}$ for 10 min before each sampling to remove memory effects (after Niinemets et al., 2011). A branch was inserted into the chamber and sealed around at the base. Plants were provided with natural PAR at 800 to 1200 µmol m$^{-2}$ s$^{-1}$ during sampling. The chamber temperature
was maintained at 30 ± 2 °C and leaf temperature was 26 ± 2 °C measured using an infra-red thermometer (Agri-Therm III™, Everest Interscience Inc, USA). Relative humidity varied from 29 to 52%. Sterile fritted glass thermal desorption (TD) tubes comprising Carboxen 1016 and Carbopack X adsorbents (Sigma Supelco®) were conditioned at 250 °C through helium purging (100 mL min⁻¹; 60 min). Volatiles were collected on to TD tubes in April and May 2013 using an oil-free pump connected to a mass flow controller (Brooks® 5850E). Chamber blank control, TD tube secondary desorption control, branch memory effect (pre-flush blank) screenings were also performed.

(b) Cuvette based leaf level sampling: A Li6400 XT portable gas exchange system was suitably modified to sample volatiles directly from the leaf cuvette onto the thermal adsorbent bed described above. The LiCor was supplied with volatile free humidified air mixed with CO₂. Within-cuvette ambient CO₂ was 400 µmol mol⁻¹; leaf temperature was 25 °C; humidity ranged from 40 to 62% and the light intensity was 1200 µmol m⁻² s⁻¹. Isoprenoids were also sampled in a low oxygen (2%), ambient CO₂ (400 µmol mol⁻¹) atmosphere generated by mixing nitrogen, high-purity O₂ and CO₂ at the required ratio and humidified to achieve 40 to 50% RH in the cuvette. Leaves were exposed to low O₂ or high O₂ for 5 to 10 min (stable readings) prior to sampling. The effect of varying oxygen exposure on photosynthesis was also studied independently (although simultaneously) on many leaves.

(c) Sampling from *E. camaldulensis* subsp. *obtusa* under controlled CO₂, O₂, temperature, and water availability: One-year-old saplings (N = 6) were maintained at 100% FC (volatiles were sampled) and then gradually dried down to 50% FC, again acclimated for 15 days (volatiles were sampled again). Before sampling, individual leaves were exposed for 10 min to five possible atmospheric CO₂ concentrations (60, 180, 400, 1000 and 1800 µmol mol⁻¹), two temperatures (28 °C and 38 °C), two O₂ concentrations (20% and 2%) and saturating light intensity (1500 µmol m⁻² s⁻¹). CO₂ compensation point remained close to 60 µmol mol⁻¹ in all treatments except in well-watered plants at 28 °C (low oxygen) where it was 10 µmol mol⁻¹. CO₂ treatment was randomised so that on a given day some leaves from different biological replicates received low CO₂ treatment while others received high CO₂ treatment to avoid either stimulation or limitation of photosynthesis.

*Thermal Desorption GC-MS analysis:* A Shimadzu GCMS-QP 2010 fitted with an auto thermal desorption system (TD-20) was used for off-line volatile analysis. Ultrapure helium (BOC) was used as the carrier gas. Isoprene and d-limonene (analytical grade, Sigma) were injected into sterile 5 L
Tedlar® bags comprising nitrogen to generate standard mixing ratios. Then, the standard mixture was adsorbed onto TD tubes (described earlier), which were used to calibrate the instrument at regular intervals. Isoprene sampled from the plant chamber was desorbed from the TD tube at 220 °C (60 mL min⁻¹ for 5 min). A 30 m, 25mm ID, 25 µm RTX-5 Sil MS (Restek) capillary column was used for GC. The temperature regime for GC run was 28 °C (3min) to 110 °C (3 min) at 5 °C min⁻¹ and finally to 180 °C at 5 °C min⁻¹. The chromatographic peaks were identified by comparing them to isoprene and monoterpane standards (α-pinene and d-limonene) and reference mass spectrographs in the NIST Standard Reference Database 1A (NIST 2008). Isoprene emission rates were calculated by utilizing sampling flow rate, total leaf area in the sampling chamber (for branches) and quantified isoprene standards.

Statistical Analysis: The statistical tests were performed using Minitab (v16 statistical package, Minitab Inc; PA, USA). Equality of means in responses within species between two treatments was analysed using paired t-tests. Differences in mean responses between two species to the same treatment were subjected to two-sample t-tests. The CO₂, O₂, temperature and drought intensity interactions were analysed using a multilevel general full factorial model with ANOVA (Montgomery, 2004). Experiments had 4 (between species) to 6 (sequential) biological replicates, 8 to 15 independent leaf level measurements (technical replicates) per treatment group.

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We thank Mr Shuangxi Zhou, Dr Christopher McRae (CBMS), Dr Ante Jerkovic (ASAM), Mr Walther Adendorff (METS), Mr Muhammad Masood (PGF) and Dr Ian Wright’s lab group. We are indebted to Dr Craig Barton and Dr Julia Cooke (University of Western Sydney) for lending us additional infra-red gas analysers and to Mr Marco Michelozzi (CNR, Italy) for preliminary screening of stored and constitutive monoterpenes. We thank Dr Roger Hiller (MQ), Dr Francesco Loreto, Dr Mauro Centritto (CNR, Italy) for critically reading the manuscript. We also thank Dr Marianne Peso, Dr Simon Griffith (MQ) for discussions and Dr Thomas Sharkey (MSU, US) for suggestions on the modelled relationship between reducing power and isoprenoid emission rates.

Figure captions:
Fig. 1: Photosynthesis and isoprenoid emission rates over a drought gradient in two eucalypts, Eucalyptus occidentalis (solid line, diamonds) and Eucalyptus camaldulensis subsp. camaldulensis (dotted line, triangles) subjected to 20% O₂ (left panel) and 2% O₂ (right panel): (A) stomatal

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conductance (B) leaf internal CO$_2$ concentration (C) transpiration rate (D) net assimilation rate (E) isoprene emission rate (F) constitutive monoterpene emission rate (each point represents $N=4$ plants; mean ± 1 SE; *$P \leq 0.05$; **$P < 0.01$; ***$P < 0.001$, comparison within species relative to 100% FC control). Note: The pronounced decline in NAR with drought in E. camaldulensis subsp. camaldulensis reflected the fact that its stomata were sensitive to soil water status, a mechanism that presumably achieves a minimum necessary transpiration rate per unit leaf area during drought stress (White et al., 2000).

Fig. 2: (A) ETR/NAR ratio and (B) Carbon cost (C) ETR/NAR ratio vs. total isoprenoid emission rates at 20% O$_2$. Relative response of linear electron transport rate (ETR) and net assimilation rate (NAR) in two eucalypts over a drought gradient. Note the greater decline in NAR in E. camaldulensis subsp. camaldulensis at 50% FC, which results in a large ETR/NAR ratio. Emission is almost twice more expensive under all conditions on carbon basis in E. camaldulensis (the drought sensitive species). The regression fits in Figure 2C encompasses data points from both species ($N=4$; mean ± 1SE for emission rates, Quadratic: $P=0.12$; Linear: $P=0.18$). If E. occidentalis were subjected to harsher droughts (% FC ≤10%), it is also likely to have followed a quadratic regression with peak emission at 25% FC and declining emission thereafter due to carbon limitation despite favourable ETR/NAR ratio.

Fig. 3: (A) Photosynthesis, (B) isoprene and (C) constitutive monoterpene emission rates response to short-term heat stress under 20% O$_2$ (left panel) and 2% O$_2$ (right panel) over a CO$_2$ concentration span (60 µmol mol$^{-1}$ to 1800 µmol mol$^{-1}$) in Eucalyptus camaldulensis subsp. obtusa (experiment 2) acclimated to well-watered condition (100% FC) and drought (50% FC) at two independent temperature treatments (28 ºC and 38 ºC) ($N=6$, means ± 1 SE).

Fig. 4: The energetic status model: The relationship between residual electron transport rate $J_r$ (not used for light-independent reactions of photosynthesis) and isoprenoid emission rate at different drought stress levels (A) E. occidentalis (solid rhomboids) and E. camaldulensis subsp. camaldulensis (solid triangles); (B) $J/V_{cmax}$ vs. isoprenoid emission rate in both species. ($N=3$ for $J_r$ and $J/V_{cmax}$; means ± SD, $N=4$ for isoprenoid emission rate; means ± 1 SE, $P<0.05$)

Fig. 5: Photorespiration, drought and isoprenoid emission: (A) $J_r$ vs. $I_c$ ($r^2=0.91$; $P=0.02$) (B) $J/V_{cmax}$ vs. $I_c$ ($r^2=0.85$; $P=0.06$) in E. camaldulensis subsp. camaldulensis (experiment 3) acclimated to well-watered (100% FC) and droughted (50% FC) conditions and exposed to three different levels
of oxygenated atmospheres. 2% and 50% O$_2$ exposure were maintained for 10 to 15 minutes before volatile sampling (N=5; means ± 1 SE). The correlation remains significant (at P<0.05) even when both isoprene and monoterpenes are considered together. (C) Response of constitutive monoterpene emission rate ($M_e$) in response to drought and varying oxygen levels. Drought (50% FC) had a significant positive impact on $M_e$ at both 2% and 20% O$_2$, which was consistent with isoprene like response of monoterpenes in *E. camaldulensis* subsp. *camaldulensis* (Fig. 1f) (N=5; means ± 1 SE; **P<0.05).

Fig. 6: Relationship between % Field Capacity and leaf water potential in *E. occidentalis* and *E. camaldulensis* subsp. *camaldulensis* (experiment 1)

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### Table 1: Major sinks for energy and reducing power generated by the light reactions of photosynthesis

<table>
<thead>
<tr>
<th>Biochemical pathway</th>
<th>Key steps</th>
<th>ATPs</th>
<th>NADPHs (or equivalents)</th>
<th>Reference</th>
<th>% Quantum Share *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calvin cycle or</td>
<td>10 RuBP + 10 CO₂ → 10 reduced C + 10 RuBP</td>
<td>30</td>
<td>20</td>
<td>Ogren, 1984</td>
<td>49 to 53</td>
</tr>
<tr>
<td>Photosynthetic Carbon Reduction cycle</td>
<td>(PCR cycle without photorespiration)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Photorespiration (PCO cycle, including RuBP recovery)</td>
<td>10 RuBP + 10 O₂ → 10 PGA → ... → 20 Gly → 10 CO₂ + 10 NH₃ + 10 Glu → ... → 10 PGly → 5 Glycerate → 10 RuBP</td>
<td>47.5 (17.5)</td>
<td>30 (10)</td>
<td>Peterhansel et al. 2010</td>
<td>23 to 29 *</td>
</tr>
<tr>
<td>Nitrate reduction † (photo-assimilation)</td>
<td>NO₃⁻ → NO₂⁻ → NH₄⁺</td>
<td>1</td>
<td>10</td>
<td>Noctor and Foyer, 1998</td>
<td>2 to 5</td>
</tr>
<tr>
<td></td>
<td>NH₄⁺ + Glu → Gln</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Gln + 2 oxyGlutamate → 2 Glu</td>
<td></td>
<td></td>
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<tr>
<td>Carbohydrate biosynthesis</td>
<td>6CO₂ → C₆H₁₂O₆ → C₁₂H₂₂O₁₂</td>
<td>19</td>
<td>12</td>
<td>Skillman, 2008</td>
<td>6 to 10</td>
</tr>
<tr>
<td>Mehler reaction ‡ (water-water cycle)</td>
<td>10 H₂O + 5 O₂ → 10 H₂O₂</td>
<td>0²</td>
<td>10</td>
<td>Heber, 2002</td>
<td>3 to 13</td>
</tr>
<tr>
<td></td>
<td>10 H₂O₂ → 20 Asc → 20 MDA + 20 H₂O</td>
<td></td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>20 MDA + 10 H₂O → 5 O₂</td>
<td></td>
<td></td>
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<tr>
<td>Other reducing sequences (include lipid biosynthesis, sulphate reduction, the MEP</td>
<td></td>
<td>20</td>
<td>10</td>
<td></td>
<td>2 to 6</td>
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<tr>
<td>pathway)</td>
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<td></td>
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<tr>
<td>TOTAL</td>
<td></td>
<td>~90</td>
<td>72</td>
<td></td>
<td>~100</td>
</tr>
</tbody>
</table>

* From Haupt-Herting and Fock (2002) and Skillman (2008): the share is determined by not only the absolute demand per pathway but also the actual instantaneous rates of each process. E.g. Nitrate reduction needs a lot of reducing power but its actual processing rate is very slow compared to core reactions of photosynthesis.

If one assumes a linear electron transport (ETR) rate of 100 µmol m⁻² s⁻¹ then approximately 50 µmol m⁻² s⁻¹ would be spent on reducing carbon (net assimilation rate ≈ 12 µmol m⁻² s⁻¹, given 4 electrons are needed per mol of CO₂ fixed). Similarly, photorespiration accounts for ~25 µmol m⁻² s⁻¹ and the remaining processes utilise 25 µmol m⁻² s⁻¹.

† Both photorespiration and nitrate reduction have access to reducing power from extra-plastid sources.

‡ PCO cycle (per se) requires 17.5 ATPs and 10 NADPH equivalents when we discount the energy consumed by PCR cycle during RuBP recovery via photorespiratory route. Therefore, the % quantum share of PCO cycle is roughly half of that of Calvin cycle.

² The Mehler reaction utilises NADH instead of NADPH. It does not consume ATPs rather adds to the pool of ATPs (Heber, 2002).
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Focus</th>
<th>Name of the species</th>
<th>Drought acclimation</th>
<th>CO₂ concentration (µmol mol⁻¹)</th>
<th>Temperature (°C)</th>
<th>Oxygen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Paired set (Fig. 1) (Fig. 2) (Fig. 4)</td>
<td>Eucalyptus occidentalis (Drought tolerant)</td>
<td>10:1</td>
<td>100% FC 70% FC (3 months) 50% FC (15 days)</td>
<td>Growing condition</td>
<td>Exposure before and during sampling</td>
</tr>
<tr>
<td></td>
<td>Effect of drought tolerance of photosynthesis on isoprenoid emission</td>
<td>Eucalyptus camaldulensis subsp. camaldulensis (Drought sensitive)</td>
<td>10:1</td>
<td>400</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>2</td>
<td>Individual species (Fig. 3)</td>
<td>Eucalyptus camaldulensis subsp. obtusa</td>
<td>2:1</td>
<td>100% FC 50% FC (1 month)</td>
<td>400</td>
<td>60, 180, 400, 1000, 1800</td>
</tr>
<tr>
<td>3</td>
<td>Individual species (Fig. 5)</td>
<td>Eucalyptus camaldulensis subsp. camaldulensis</td>
<td>10:1</td>
<td>100% FC 50% FC (10 days)</td>
<td>400</td>
<td>400</td>
</tr>
</tbody>
</table>
Fig. 1: Photosynthesis and isoprenoid emission rates over a drought gradient in two eucalypts.
Fig. 2: (A) ETR/NAR ratio and (B) Carbon cost (C) ETR/NAR ratio vs. total isoprenoid emission rates.
Figure 3

(A) Photosynthesis, (B) isoprene and (C) constitutive monoterpene emission rates response to short-term heat stress in *Eucalyptus camaldulensis* subsp. *obtusa*.
Fig. 4: The energetic status model
Fig. 5: Photorespiration, drought and isoprenoid emission