On-line analysis of the $^{13}CO_2$ labelling reveals *in vivo* the chloroplastic and extrachloroplastic carbon contribution to isoprene synthesis during drought stress in *Populus nigra* leaves

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Isoprene (2-methyl-1,3-butadiene) is the most abundant hydrocarbon emitted by various plant species (Kesselmeier and Staudt, 1999). The main carbon source for isoprene synthesis is the photosynthetic carbon reduction pathway. This has been demonstrated by fast and quasi-complete ^{13}C labelling of the five carbon atoms of isoprene when using $^{13}CO_2$ in the air(Delwiche & Sharkey, 1993). The unlabelled isoprene fraction (10-20% under non-stressed conditions) suggests however that some extrachloroplastic carbon sources also produce this compound. Environmental stress conditions strongly influence, and often stimulate, the metabolism and emission of isoprene. Drought stress in particular seems to have limited effect on isoprene emission, even when photosynthetic rates are severely inhibited (Sharkey and Loreto, 1993). We addressed the question whether extrachloroplastic carbon sources are responsible for the sustained emission of isoprene under drought stress conditions.

We used proton-transfer-reaction mass spectrometry (PTR-MS) (Lindinger et al., 1998) to record the appearance of individually ^{13}C-labelled atoms in the unfragmented isoprene molecule following exposure to air containing $^{13}CO_2$ under severe drought stress conditions. Furthermore, we monitored ^{13}C labelling of the C3 fragment of isoprene which should contain C2 mojety derived from extrachloroplastic carbon (Karl et al., 2002). Even when carbon fixation by photosynthesis was compensated by respiratory processes at a severe drought stress stage, isoprene was emitted at a 30% rate of the emission observed in irrigated controls. However, only 20-50% of the emitted isoprene was labelled by $^{13}CO_2$. These results indicate that even under severe drought stress conditions, photosynthesis continues to feed carbon for isoprene synthesis. The extrachloroplastic carbon source is insensitive to stress or may increasingly supply carbon to isoprene when the stress is severe. From the C3 fragment labelling analysis we speculate that this extrachloroplastic precursor may be cytosolic pyruvate/phosphoenolpyruvate equivalents transported into the chloroplast.