detailed surveys of hydrothermal sites and additional geophysical studies.

One of the factors driving the continuing search for hydrothermal vents on the Gakkel ridge is the unknown make-up of the chemosynthetic faunal communities that surround them. The fauna inhabiting known Atlantic and Pacific vent sites are markedly different from each other, and also exhibit distinct biogeographical differences within each of these oceans[3,27]. There is no modern deep-water (ridge depth) connection between the Gakkel ridge and other parts of the mid-ocean ridge system south of Iceland. Current knowledge of the tectonic history of the Arctic Ocean[28,29] suggests that there has been no such connection between the Arctic and the other major ocean basins during its history. Therefore it is likely that new species of vent biota, which have evolved in isolation from those in other oceans, will be discovered at hydrothermal sites on the Gakkel ridge[3]. Future studies that locate these vent sites, and return images and samples from them, will provide insights into the evolution of vent organisms and the pathways by which they migrated into these sites. □

Received 1 October; accepted 26 November 2002; doi:10.1038/nature01351.

1. Chen, Y. J. in *Ophiolites and Oceanic Crust: New Insights from Field Studies and the Ocean Drilling Program* (eds Dilek, Y., Moores, E. M., Elthon, D. & Nicolas, A.) 161–179 (Geological Society of America, Boulder, Colorado, 2000).
2. Baker, E. T., German, C. R. & Elderfield, H. in *Seafloor Hydrothermal Systems: Physical, Chemical, Biological, and Geological Interactions* (eds Humphris, S. E., Zierenberg, R. A., Mullineaux, L. S. & Thomson, R. E.) 47–71 (American Geophysical Union, Washington DC, 1995).
3. Van Dover, C. L., German, C. R., Speer, K. G., Parson, L. M. & Vrijenhoek, R. C. Evolution and biogeography of deep-sea vent and seep invertebrates. *Science* **295,** 1253–1257 (2002).
4. Baker, E. T., Chen, Y. J. & Phipps Morgan, J. The relationship between near-axis hydrothermal cooling and the spreading rate of mid-ocean ridges. *Earth Planet. Sci. Lett.* **142,** 137–145 (1996).
5. German, C. R. & Parson, L. M. Distributions of hydrothermal activity along the Mid-Atlantic Ridge: interplay of magmatic and tectonic controls. *Earth Planet. Sci. Lett.* **160,** 327–341 (1998).
6. Lupton, J. E., Delaney, J. R., Johnson, H. P. & Tivey, M. K. Entrainment and vertical transport of deep-ocean water by buoyant hydrothermal plumes. *Nature* **316,** 621–623 (1985).
7. Klinkhammer, G., Rona, P., Greaves, M. & Elderfield, H. Hydrothermal manganese plumes in the Mid-Atlantic Ridge rift valley. *Nature* **314,** 727–731 (1985).
8. Scheirer, D. S., Baker, E. T. & Johnson, K. T. M. Detection of hydrothermal plumes along the Southeast Indian Ridge near the Amsterdam-St. Paul Plateau. *Geophys. Res. Lett.* **25,** 97–100 (1998).
9. Charlou, J. L. & Donval, J. P. Hydrothermal methane venting between 12°N and 26°N along the Mid-Atlantic Ridge. *J. Geophys. Res.* **98,** 9625–9642 (1993).
10. Baker, E. T. & Milburn, H. B. MAPR: A new instrument for hydrothermal plume mapping. *Ridge Events* **8,** 23–25 (1997).
11. Bach, W., Banerjee, N. R., Dick, H. J. B. & Baker, E. T. Discovery of ancient and active hydrothermal systems along the ultra-slow spreading Southwest Indian Ridge 10°-16°E. *Geochem. Geophys. Geosyst.* **3,** 101029/2001GC000279 (2002).
12. Baker, E. T., Cormier, M.-H., Langmuir, C. H. & Zavala, K. Hydrothermal plumes along segments of contrasting magmatic influence, 15°20′N-18°30′N, East Pacific Rise: Influence of axial faulting. *Geochem. Geophys. Geosyst.* **2,** 2000GC000165 (2001).
13. Müller, C. & Jokat, W. Seismic evidence for volcanic activity discovered in central Arctic. *Eos* **81,** 265–269 (2000).
14. Tolstoy, M., Bohnenstiehl, D. R., Edwards, M. H. & Kurras, G. J. Seismic character of volcanic activity at the ultraslow-spreading Gakkel Ridge. *Geology* **29,** 1139–1142 (2001).
15. Edwards, M. H. *et al.* Evidence of recent volcanic activity on the ultraslow-spreading Gakkel ridge. *Nature* **409,** 808–812 (2001).
16. Speer, K. G. & Rona, P. A. A model of an Atlantic and Pacific hydrothermal plume. *J. Geophys. Res.* **94,** 6213–6220 (1989).
17. German, C. R., Parson, L. M. & the HEAT Scientific Team. Hydrothermal exploration near the Azores Triple Junction: tectonic control of venting at slow-spreading ridges? *Earth Planet. Sci. Lett.* **138,** 93–104 (1996).
18. German, C. R., Baker, E. T., Mevel, C., Tamaki, K. & the FUJI Scientific Team. Hydrothermal activity along the southwest Indian Ridge. *Nature* **395,** 490–493 (1998).
19. German, C. R. *et al.* Hydrothermal activity on the Reykjanes Ridge: the Steinahóll vent-field at 63°06′N. *Earth Planet. Sci. Lett.* **121,** 647–654 (1994).
20. Kleinrock, M. C. & Humphris, S. E. Structural control on sea-floor hydrothermal activity at the TAG active mound. *Nature* **382,** 149–153 (1996).
21. Fouquet, Y. *et al.* FLORES diving cruise with the Nautile near the Azores—First dives on the Rainbow field: hydrothermal seawater/mantle interaction. *InterRidge News* **7,** 24–28 (1998).
22. Charlou, J. L. *et al.* Intense CH_4 plumes generated by serpentinization of ultramafic rocks at the intersection of the 15°20′N fracture zone and the Mid-Atlantic Ridge. *Geochim. Cosmochim. Acta* **62,** 2323–2333 (1998).
23. Kelley, D. S. *et al.* An off-axis hydrothermal vent field near the Mid-Atlantic Ridge at 30° N. *Nature* **412,** 145–149 (2001).
24. Coakley, B. J. & Cochran, J. R. Gravity evidence of very thin crust at the Gakkel Ridge (Arctic Ocean). *Earth Planet. Sci. Lett.* **162,** 81–95 (1998).
25. Bown, J. W. & White, R. S. Variation with spreading rate of oceanic crustal thickness and geochemistry. *Earth Planet. Sci. Lett.* **121,** 435–449 (1994).
26. Kurras, G. J. *et al.* Axial valley morphology of the Gakkel Ridge [8°W-88°E]: Seabeam and Hydrosweep bathymetry from the Arctic Mid-Ocean Ridge Expedition (AMORE 2001). *Eos* **82** (Fall Meeting Suppl), T11B-0855 (2001).
27. Tunnicliffe, V. & Fowler, C. M. R. Influence of sea-floor spreading on the global hydrothermal vent fauna. *Nature* **378,** 531–533 (1996).
28. Johnson, G. L., Pogrebitsky, J. & Macnab, R. in *The Polar Oceans and Their Role in Shaping the Global Environment* (eds Johannessen, R. E., Muench, R. D. & Overland, J. E.) 285–294 (American Geophysical Union, Washington DC, 1994).
29. Lawver, L. A., Muller, R. D., Srivastava, S. P. & Roest, W. in *Geological History of the Polar Oceans: Arctic versus Antarctica* (eds Bleil, U. & Thiede, J.) 29–62 (Kluwer Academic, Dordrecht, 1990).
30. Baker, E. T., Tennant, D. A., Feely, R. A., Lebon, G. T. & Walker, S. L. Field and laboratory studies on the effect of particle size and composition on optical backscattering measurements in hydrothermal plumes. *Deep-Sea Res. I* **48,** 593–604 (2001).

Supplementary Information accompanies the paper on *Nature*'s website (**http://www.nature.com/nature**).

Acknowledgements We thank all the members of the AMORE science party for their assistance, and the officers and crews of the USCGC *Healy* and PFS *Polarstern* for their logistical and technical support. We also thank S. Walker for assistance with MAPR support and Fig. 3. This work was supported by the US National Science Foundation, the US NOAA Vents Program, a Heisenberg fellowship to J.E.S. from the Deutscheforschungsgemeinschaft, and W. S. Gardner and the University of Texas at Austin.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to H.N.E. (e-mail: edmonds@utmsi.utexas.edu).

Increased CO_2 uncouples growth from isoprene emission in an agriforest ecosystem

Todd N. Rosenstiel*†‡, Mark J. Potosnak†§||, Kevin L. Griffin§, Ray Fall‡¶ & Russell K. Monson*‡

* *Department of Environmental, Population, and Organismic Biology,*
‡ *Cooperative Institute for Research in Environmental Sciences,*
¶ *Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309, USA*
§ *Lamont-Doherty Earth Observatory, Columbia University, Palisades, New York 10964, USA*
† *These authors contributed equally to this work*

The emission of isoprene from the leaves of forest trees is a fundamental component of biosphere–atmosphere interactions, controlling many aspects of photochemistry in the lower atmosphere[1–3]. As almost all commercial agriforest species emit high levels of isoprene[4], proliferation of agriforest plantations has significant potential to increase regional ozone pollution[5–7] and enhance the lifetime of methane[8], an important determinant of global climate. Here we show that growth of an intact *Populus deltoides* plantation under increased CO_2 (800 μmol mol^{-1} and 1,200 μmol mol^{-1}) reduced ecosystem isoprene production by 21% and 41%, while above-ground biomass accumulation was enhanced by 60% and 82%, respectively. Exposure to increased CO_2 significantly reduced the cellular content of dimethylallyl diphosphate, the substrate for isoprene synthesis, in both leaves and leaf protoplasts. We identify intracellular metabolic competition for phosphoenolpyruvate as a possible control point in explaining the suppression of isoprene emission under increased CO_2. Our results highlight the potential for uncoupling isoprene emission from biomass accumulation in an agriforest species, and show that negative air-quality effects of proliferating agriforests may be offset by increases in CO_2.

|| Present address: National Center for Atmospheric Research, Boulder, Colorado 80305, USA.

Unlike other crops, almost all fast-growing agriforest species, including *Acacia* (tropical), *Eucalyptus* (tropical) and *Populus* (temperate), emit significant quantities of isoprene (2-methyl-1,3-butadiene)[4,9], a highly reactive hydrocarbon. Afforestation with such fast-growing trees has been suggested as a way to ameliorate increases in atmospheric CO_2 concentration[10], [CO_2], and plantation forests will undoubtedly be important in supplying future global demand for wood[11]. When such plantations occur on large spatial scales, the effects on atmospheric chemistry can be marked[12]. As a result, the continued expansion of agriforest plantations (10.5 Mha yr^{-1} at present)[13] has the potential to affect significantly the oxidative behaviour of the atmosphere, in turn negatively affecting local air quality[5,6], perturbing regional biogeochemical cycles[3] and further contributing to climate forcing by enhancing the lifetime of tropospheric methane[8]. However, realistic estimates of regional isoprene emission are difficult to obtain, and we also lack an adequate understanding of the biochemical mechanisms that may couple isoprene emission to increased [CO_2] and associated global climate change.

To examine the effect of [CO_2] on isoprene emission of an agriforest species, we established three cottonwood plantations (*Populus deltoides* Bartr.), from approximately 50 trees each, in the forestry section of Columbia University's Biosphere 2 Center, a large controlled-environment research facility[14]. The poplar clone used was obtained from Westvaco Corporation and is currently used as planting stock for *Populus* agriforests in the south-eastern United States. Poplar clones were planted in 1998 in three large replicate mesocosms. [CO_2] set points within the three mesocosms have been maintained at 430 μmol mol^{-1} (p.p.m.), 800 p.p.m. and 1,200 p.p.m. since May 2000. By the end of the growing season (day 341), canopy height ranged from 7.5–8.1 m (approximately one-half mesocosm height).

The semi-closed nature of the Biosphere 2 facility (see Methods) allowed a continuous determination of net ecosystem isoprene production. Figure 1 shows the typical day-to-day variability and pattern of isoprene emission in the three mesocosms. All three mesocosms displayed the well-characterized light-dependent diurnal pattern of isoprene emission[15], with maximal emissions occurring at roughly midday. Compared with the current [CO_2] of 430 p.p.m., growth at increased [CO_2] resulted in marked decreases in the daily release of isoprene (Fig. 1). Here we report the effects of atmospheric [CO_2] on whole-ecosystem isoprene emissions; the results are similar to previous reports of leaf-level suppression of isoprene emission in *Populus*[16] in response to increased [CO_2].

When integrated over the 2000 growing season, net ecosystem isoprene production was reduced at increased [CO_2] by 21% and 41%, respectively (Table 1). However, reductions in ecosystem isoprene production were not a consequence of decreased productivity. Total above-ground plant biomass harvested at the end of the growing season increased by 60% and 82% with increased [CO_2] (Table 1). We also measured leaf-level photosynthesis rates throughout the growing season, which were generally enhanced under increased [CO_2] (data not shown). Throughout the course of the experiment, the poplar leaves displayed no indication of photosynthetic downregulation at either 800 or 1,200 p.p.m. CO_2. Instead, the biomass data suggests a significant degree of CO_2 fertilization of this rapidly growing species, supporting numerous other studies suggesting that the productivity and capacity to sequester carbon in *Populus* sp. may benefit from enhanced [CO_2][17]. To summarize the mesocosm results, it appears that the percentage of fixed CO_2 converted to isoprene is decreased by CO_2 fertilization.

To study the biochemical nature of the isoprene suppression, we measured the content of dimethylallyl diphosphate (DMAPP) in leaves growing in each of the three mesocosms. DMAPP is the immediate precursor of isoprene biosynthesis, and the regulation of its production in plant chloroplasts has been implicated in the control of isoprene emission[18]. Isoprene-emission rates were determined on leaves ($n = 6$–12) from each mesocosm in the middle of the photoperiod and subsequently harvested for DMAPP analysis. Both isoprene-emission rates (normalized to leaf area) and DMAPP content were significantly reduced at increased [CO_2], and there was a significant positive correlation between DMAPP content and

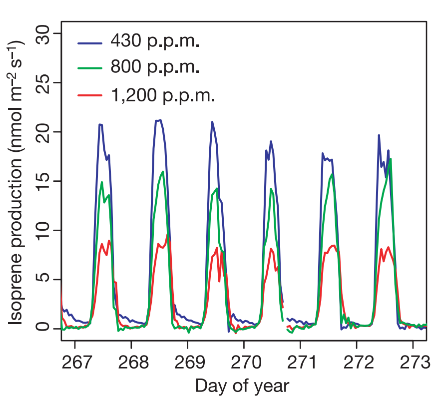

Figure 1 Net ecosystem isoprene production for *Populus deltoides* plantations. Data is shown for growth at three different concentrations of CO_2 at the Biosphere 2 facility. The figure depicts six representative days during the 2000 growing season. Isoprene production is expressed per ecosystem area.

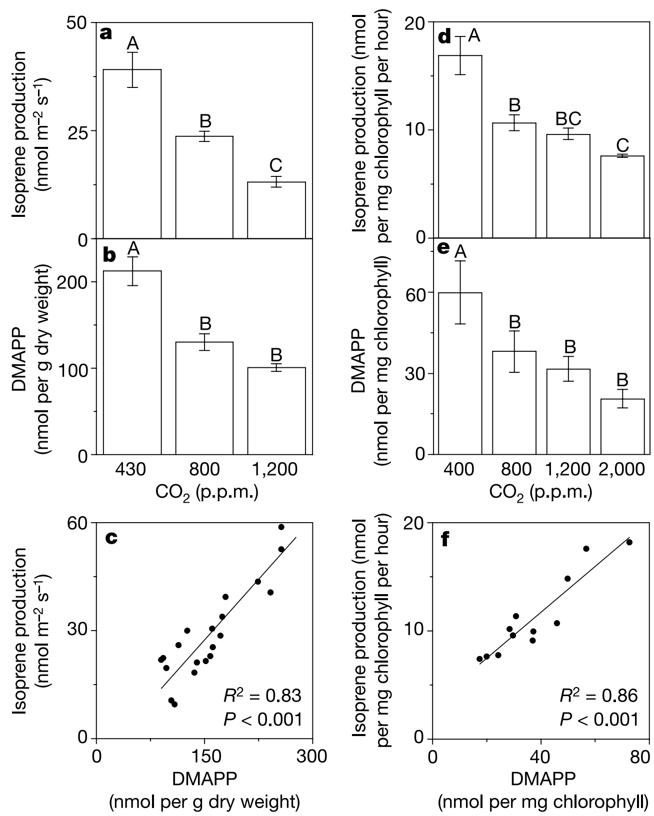

Figure 2 Isoprene production and dimethylallyl diphosphate (DMAPP) content in leaves and protoplasts of *P. deltoides*. **a**–**c**, Isoprene production (**a**), DMAPP content (**b**) and their relationship with a fitted linear regression (**c**) from leaves collected at the Biosphere 2 facility. Values represent the mean ± s.e.m. ($n = 6$–8). **d**–**f** Isoprene production (**d**), DMAPP content (**e**) and their relationship with a fitted linear regression (**f**) from protoplasts incubated under four concentrations of CO_2. Values represent the mean ± s.e.m. ($n = 3$). Within a panel, means with the same letter are not significantly different ($P < 0.05$).

Table 1 **Isoprene production and biomass accumulation at varying [CO_2]**

[CO_2] (p.p.m.)	Isoprene production (mmol m^{-2})	Biomass (g m^{-2})
430	79.5	668
800	61.4	1,070
1,200	46.9	1,220

Values for the net ecosystem isoprene production and the above-ground biomass accumulation are expressed per ecosystem area. Measures were obtained for three *Populus deltoides* plantations grown at three concentrations of CO_2 ([CO_2]) in the Biosphere 2 facility during the 2000 growing season.

isoprene emission (Fig. 2a–c). These data indicate that controls over DMAPP synthesis underlie the suppression of isoprene emission at increased [CO_2].

To further examine this possibility, we developed an independent experimental system using isolated protoplasts. Intact, isoprene-emitting protoplasts were isolated from leaves of the same clones of *P. deltoides* Bartr. growing in greenhouses at the University of Colorado, Boulder. When incubated under increased [CO_2], protoplasts showed the same suppression of isoprene production and DMAPP content (Fig. 2d–f), and the same strong positive correlation between DMAPP content and isoprene production as leaves grown under increased [CO_2]. Isoprene emission from protoplasts was found to be especially sensitive to inhibition by $NaHCO_3$ (Fig. 3), an alternative CO_2 source. Once again, the suppression of isoprene emission was strongly positively correlated with the cellular concentration of DMAPP. Taken together, results from both whole leaves and isolated protoplasts strongly support the hypothesis that the suppression of isoprene emission under increased [CO_2] involves substrate-level regulation and is a direct result of reduced DMAPP production.

The controls over DMAPP synthesis in chloroplasts are poorly understood[19]. However, recent results indicate that a significant fraction of carbon entering the chloroplastic DMAPP pool is extra-chloroplastic in origin[20], and may arise from cytosolic phosphoenolpyruvate (PEP). If so, then competition for PEP in the plant cell cytosol could have an impact on isoprene emission in the chloroplast. We proposed that increasing cellular [HCO_3^-], in response to increasing [CO_2], might directly reduce cytosolic PEP concentrations by stimulating the activity of cytosolic PEP-carboxylase (PEPC). PEPC, a ubiquitous plant enzyme, catalyses the carboxylation of PEP to oxaloacetic acid for anaplerotic reactions in plant cells[21], and is present with high activity in the leaves of *Populus* (data not shown). To test the role of PEPC in influencing isoprene formation, we fed two PEPC inhibitors to excised leaves of *P. deltoides* (Fig. 4). Addition of either diethyloxalacetate (DOA) or 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)-propenoate (DCDP, see Methods) enhanced the rates of isoprene emission, supporting the hypothesis that isoprene emission is responsive to the availability of cytosolic PEP. These general results were replicated several times, and experiments with *Eucalyptus* and northern red oak, both isoprene emitters, yielded similar results (data not shown). The increase in isoprene emission occurred despite variable impacts on photosynthesis. When PEPC was assayed in whole-leaf extracts, addition of either DOA or DCDP led to a significant inhibition of activity (data not shown).

It should be noted that growth in increased [CO_2] has been shown to increase both leaf mitochondrial number[22] and the rate of leaf respiration in the light[23]. Presumably, increased conversion of PEP to pyruvate must occur under increased [CO_2] to provide the necessary substrate for increased mitochondrial respiration during the day, further reducing the availability of cytosolic PEP for other cellular processes. We speculate that suppression of isoprene emission reflects a more fundamental metabolic response of plants to increases in [CO_2]—namely, altered partitioning of PEP between mitochondrial and chloroplastic processes. In addition, this dynamic exchange between the mitochondria and the chloroplast may help to explain why the CO_2 effect on isoprene emission has been variable[16].

The emission of isoprene from agriforest species represents a significant unintended consequence of agriforest cultivation. As short-rotation agriforests provide an increasing array of human services—including lumber, pulp and paper production, biofuels for renewable energy and, potentially, carbon sequestration[10,11]—the continued widespread establishment of these managed ecosystems is likely to influence the continued rise in tropospheric ozone[7,8] as well as the sustainability of the oxidative power of the atmosphere[24]. Although isoprene emission is generally considered to be closely linked to photosynthetic metabolism[25], results from this study show that leaf development under increased [CO_2] reduced isoprene emission, despite concomitant increases in both photosynthesis and biomass accumulation. The identification of intracellular metabolic competition for PEP provides a mechanism for reconciling the decreased chloroplastic isoprene emission with the, presumably, increased rates of mitochondrial respiration necessary for biomass accumulation. The results from this study confirm

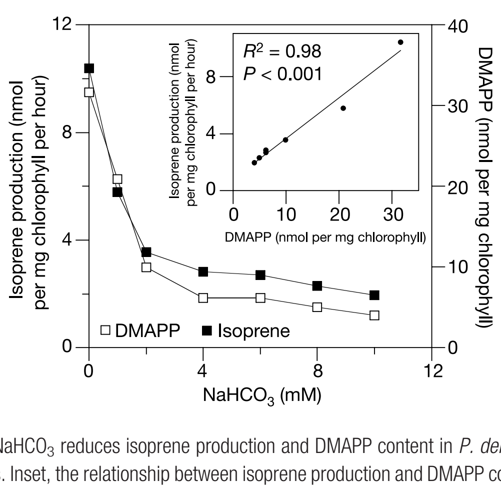

Figure 3 $NaHCO_3$ reduces isoprene production and DMAPP content in *P. deltoides* protoplasts. Inset, the relationship between isoprene production and DMAPP content with a fitted linear regression.

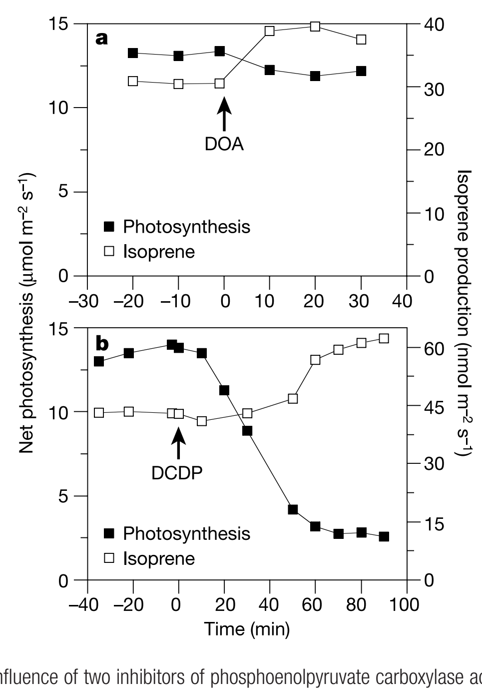

Figure 4 Influence of two inhibitors of phosphoenolpyruvate carboxylase activity on isoprene production and net photosynthesis in excised leaves of *P. deltoides*. **a**, Diethyloxalacetate (DOA; 100 μM) **b**, 3,3-dichloro-2-(dihydroxyphosphinoylmethyl)-propenoate (DCDP; 4 mM). Arrows and time zero indicate the application of inhibitor.

experimentally in an intact *Populus* ecosystem that increased [CO_2] cannot only enhance biomass accumulation in a short-rotation agriforest, but also reduce net ecosystem isoprene production. So the negative air-quality impacts (that is, isoprene emission) of proliferating agriforests may be partially offset by increases in global [CO_2]. □

Methods

Biosphere 2 experiment

This experiment was conducted in the 2,000 m^2 forestry section of Columbia University's Biosphere 2 Center (Oracle, Arizona, USA)[14]. The forestry section is physically isolated from the remainder of Biosphere 2, and has independent climate and CO_2 control; it is subdivided into three roughly equal 12,000 m^3 mesocosms. Outside air is constantly exchanged with a set of push–pull fans, and [CO_2] within each bay is maintained at set points by adding pure CO_2 from a liquid storage tank. The mesocosms are subjected to the light regimes of a temperate desert region, attenuated by the glass and steel structure. An automated climate-control system maintains temperature, dew point and soil moisture (for further details, see ref. 26). Cottonwood (*P. deltoides* Bartr.) clones (S7c8-day neutral) used in this experiment were obtained from a fibre production farm (Westvaco Corporation); they are adapted to the lower Brazos River, Texas. Clones were planted in 1998 and coppiced at the end of each growing season. Each mesocosm contained 43–47 trees. We calculated hourly ecosystem isoprene production from mesocosm isoprene concentrations measured with a Fast Isoprene Sensor (Hills Scientific); volumetric exchange rates and leak rates were determined from SF6 injection, using ten-day averages to fill data voids.

Leaf photosynthesis and isoprene measurements

Leaf-level gas-exchange measurements were made with a portable photosynthesis system (Li-6400, Li-Cor Inc.). All measurements were made on attached leaves at 30 °C and 1,000 μmol photons m^{-2} s^{-1}, and with growth [CO_2] at 430 p.p.m., 800 p.p.m. or 1,200 p.p.m. within Biosphere 2; in the case of the inhibitor experiments, [CO_2] was at 360 p.p.m. for detached leaves from trees growing at ambient [CO_2] in greenhouses at the University of Colorado. To determine leaf-level isoprene-emission rates, a small sample of air exiting the cuvette was injected into a gas chromatograph and measured with either photo-ionization or reduction gas detection[15].

Protoplast experiments

Protoplasts were isolated from young leaves of *P. deltoides* Bartr. as described in ref. 27, with slight modification to enhance yield[28]. To determine isoprene production, protoplasts, equivalent to 25 μg of chlorophyll, were incubated in small vials containing 0.7 M sorbitol, 50 mM HEPES (pH = 7.0), 2 mM $MgCl_2$ and 1 mM $CaCl_2$, and head-space isoprene concentrations were determined by gas chromatography (reduction gas detection) after a 30–60-min incubation at 1,000 μmol m^{-2} s^{-1} at 25 °C. CO_2 was provided as either CO_2 gas occupying the vial headspace or as $NaHCO_3$.

DMAPP analysis

DMAPP content was determined as isoprene released following acidification of lyophilized leaf material[18], or after rapid acidification of intact protoplasts and subsequent head-space analysis.

PEPC inhibitors

Stock solutions of DOA and DCDP were prepared as described[29,30] and supplied to the cut petiole (final concentrations: 100 μM DOA and 4 mM DCDP).

Received 14 October; accepted 15 November 2002; doi:10.1038/nature01312.
Published online 5 January 2003.

1. Fehsenfeld, F. C. *et al.* Emissions of volatile organic compounds from vegetation and the implications for atmospheric chemistry. *Glob. Biogeochem. Cycles* **6,** 389–430 (1992).
2. Fuentes, J. D. *et al.* Biogenic hydrocarbons in the atmospheric boundary layer: a review. *Bull. Am. Meteorol. Soc.* **81,** 1537–1575 (2000).
3. Monson, R. K. & Holland, E. A. Biospheric trace gas fluxes and their control over tropospheric chemistry. *Annu. Rev. Ecol. Syst.* **32,** 547–576 (2001).
4. Harley, P. C., Monson, R. K. & Lerdau, M. T. Ecological and evolutionary aspects of isoprene emission from plants. *Oecologia* **118,** 109–123 (1999).
5. Trainer, M. *et al.* Models and observations of the impact of natural hydrocarbons on rural ozone. *Nature* **329,** 705–707 (1987).
6. Chameides, W. L., Lindsay, R. W., Richardson, J. & Kiang, C. S. The role of biogenic hydrocarbons in urban photochemical smog: Atlanta as a case study. *Science* **241,** 1473–1475 (1988).
7. Pierce, T. *et al.* Influence of increased isoprene emissions on regional ozone modeling. *J. Geophys. Res.* **103,** 25611–25629 (1999).
8. Poisson, N., Kanakidou, M. & Crutzen, P. J. Impact of non-methane hydrocarbons on tropospheric chemistry and the oxidizing power of the global troposphere: three-dimensional modeling results. *J. Atmos. Chem.* **36,** 157–230 (2000).
9. Lerdau, M. & Slobodkin, L. Trace gas emissions and species-dependent ecosystem services. *Trends Ecol. Evol.* **17,** 309–312 (2002).
10. Brown, S., Sathaye, J., Cannell, M. & Kauppi, P. in *Climate Change in 1995—Impacts, Adaptations and Mitigation of Climate Change: Scientific–Technical Analyses. Contribution of Working Group II to the Second Assessment Report of the Intergovernmental Panel on Climate Change* (eds Watson, R. T., Zinowera, M. C. & Moss, R. H.) 776–797 (Cambridge Univ. Press, Cambridge, 1996).
11. Fenning, T. M. & Gershenzon, J. Where will the wood come from? Plantation forests and the role of biotechnology. *Trends Biotechnol.* **20,** 291–296 (2002).
12. Muller, J. F. Geographical distribution and seasonal variation of surface emissions and deposition velocities of atmospheric trace gases. *J. Geophys. Res.* **97,** 3787–3804 (1992).
13. *Forest Products 1991-1995*. FAO Forestry Series No. 30, FAO Statistic Series No. 137/(Food and Agriculture Organization of the United Nations, Rome, 1995).
14. Marino, B. D. V. & Odum, H. T. *Biosphere 2 Research Past and Present* (Elsevier, New York, 1999).
15. Harley, P., Guenther, A. & Zimmerman, P. Environmental controls over isoprene emission in deciduous oak canopies. *Tree Physiol.* **17,** 705–714 (1997).
16. Sharkey, T. D., Loreto, F. & Delwiche, F. High carbon dioxide and sun/shade effects on isoprene emission from oak and aspen tree leaves. *Plant/Cell Environ.* **14,** 333–338 (1991).
17. Gielen, B. & Ceulemans, R. The likely impact of rising atmospheric CO_2 on natural and managed *Populus*: a literature review. *Environ. Pollut.* **115,** 335–338 (2001).
18. Rosenstiel, T. N., Fisher, A. J., Fall, R. & Monson, R. K. Differential accumulation of dimethylallyl-diphosphate in leaves and needles of isoprene- and methylbutenol-emitting and non-emitting species. *Plant Physiol.* **129,** 1276–1284 (2002).
19. Lichtenthaler, H. K. The 1-deoxy-D-xylulose-5-phosphate pathway of isoprenoid biosynthesis in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **50,** 47–65 (1999).
20. Karl, T. *et al.* On line analysis of the $^{13}CO_2$ labeling of leaf isoprene suggests multiple subcellular origins of isoprene precursors. *Planta* **215,** 894–905 (2002).
21. Chollet, R., Vidal, J. & O'Leary, M. H. Phosphoenolpyruvate carboxylase: a ubiquitous highly regulated enzyme in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47,** 273–298 (1996).
22. Griffin, K. L. *et al.* Plant growth in elevated CO_2 alters mitochondrial number and chloroplast fine structure. *Proc. Natl Acad. Sci. USA* **98,** 2473–2478 (2001).
23. Wang, X., Lewis, J. D., Tissue, D. T., Seeman, J. R. & Griffin, K. L. Effects of elevated atmospheric CO_2 concentration of leaf dark respiration of *Xanthium strumarium* in light and in darkness. *Proc. Natl Acad. Sci. USA* **98,** 2479–2484 (2001).
24. Crutzen, P. J., Lawrence, M. G. & Poschl, U. On the background photochemistry of tropospheric ozone. *Tellus A* **51,** 123–146 (1999).
25. Sharkey, T. D. & Yeh, S. Isoprene emission from plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52,** 407–436 (2001).
26. Griffin, K. L. *et al.* Leaf respiration is differentially affected by leaf vs. stand-level night-time warming. *Glob. Change Biol.* **8,** 479–485 (2002).
27. Sanadze, G. A., Mgaloblishvili, M. P. & Dalakishvili, K. G. Action of cerulenin, organophosphorous compounds, and synthetic analogs of carbohydrates on CO_2 assimilation and the isoprene effect in isolated Poplar protoplasts. *Sov. Plant Physiol.* **37,** 1–5 (1990).
28. Edwards, G. E., Robinson, S. P., Tyler, N. J. C. & Walker, D. A. A requirement for chelation in obtaining functional chloroplasts of Sunflower and Wheat. *Arch. Biochem. Biophys.* **190,** 421–433 (1978).
29. Magin, N. C., Cooley, B. A., Reiskind, J. B. & Bowes, G. Regulation and localization of key enzymes during the induction of Kranz-less, C4-type photosynthesis in *Hydrilla verticillata*. *Plant Physiol.* **115,** 1681–1689 (1997).
30. Jenkins, C. L. D., Furbank, R. T. & Hatch, M. D. Inorganic carbon diffusion between C4 mesophyll and bundle sheath cells. *Plant Physiol.* **91,** 1356–1363 (1989).

Acknowledgements We thank R. Murthy for assistance at the Biosphere 2 Center, W. Kahtri for technical assistance, and C. Jenkins for supplying DCDP. This work was supported in part by grants from the National Science Foundation (NSF), the Department of Energy and the Packard Foundations to R.F., R.K.M. and K.L.G., an NSF fellowship to M.J.P., a Cooperative Institute for Research in Environmental Sciences fellowship to T.N.R., and a Columbia University Biosphere 2 grant to R.K.M.

Competing interests statement The authors declare that they have no competing financial interests.

Correspondence and requests for materials should be addressed to T.N.R. (e-mail: todd.rosenstiel@colorado.edu).

Speciation along environmental gradients

Michael Doebeli* & Ulf Dieckmann†

** Departments of Zoology and Mathematics, University of British Columbia, Vancouver, Canada V6T 1Z4*
† Adaptive Dynamics Network, International Institute for Applied Systems Analysis, A-2361 Laxenburg, Austria

Traditional discussions of speciation are based on geographical patterns of species ranges[1,2]. In allopatric speciation, long-term geographical isolation generates reproductively isolated and spatially segregated descendant species[1,3]. In the absence of geographical barriers, diversification is hindered by gene flow[1,3,4]. Yet a growing body of phylogenetic and experimental data suggests that closely related species often occur in sympatry