Emission of Isoprene from Salt-Stressed *Eucalyptus globulus* Leaves[1]

Francesco Loreto* and Sebastiano Delfine[2]

Consiglio Nazionale delle Ricerche, Istituto di Biochimica ed Ecofisiologia Vegetali, Via Salaria Km 29,300, 00016 Monterotondo Scalo, Rome, Italy

Eucalyptus spp. are among the highest isoprene emitting plants. In the Mediterranean area these plants are often cultivated along the seashore and cope with recurrent salt stress. Transient salinity may severely but reversibly reduce photosynthesis and stomatal conductance of *Eucalyptus globulus* leaves but the effect on isoprene emission is not significant. When the stress is relieved, a burst of isoprene emission occurs, simultaneously with the recovery of photosynthetic performance. Later on, photosynthesis, stomatal conductance, and isoprene emission decay, probably because of the onset of leaf senescence. Isoprene emission is not remarkably affected by the stress at different light intensities, CO_2 concentrations, and leaf temperatures. When CO_2 was removed and O_2 was lowered to inhibit both photosynthesis and photorespiration, we found that the residual emission is actually higher in salt-stressed leaves than in controls. This stimulation is particularly evident at high-light intensities and high temperatures. The maximum emission occurs at 40°C in both salt-stressed and control leaves sampled in ambient air and in control leaves sampled in CO_2-free and low-O_2 air. However, the maximum emission occurs at 45°C in salt-stressed leaves sampled in CO_2-free and low-O_2 air. Our results suggest the activation of alternative non-photosynthetic pathways of isoprene synthesis in salt-stressed leaves and perhaps in general in leaves exposed to stress conditions. The temperature dependence indicates that this alternative synthesis is also under enzymatic control. If this alternative synthesis still occurs in the chloroplasts, it may involve a thylakoid-bound isoprene synthase.

The ecophysiology of isoprene emission from plants has been studied for years (Jones and Rasmussen, 1975). These studies unraveled the environmental control over the emission (Monson and Fall, 1989; Loreto and Sharkey, 1990; Fall and Monson, 1992; Sharkey and Loreto, 1993; Monson et al., 1994) and helped in elucidating the biochemical pathway of isoprene formation (Sharkey et al., 1991), which more recently has been conclusively identified (Lichtenthaler et al., 1997). This information is in turn useful for predicting the emission of this highly reactive biogenic compound and the consequent potential formation of tropospheric ozone (Guenther et al., 1991, 1995).

The genus *Eucalyptus* is one of the highest emitters of isoprene (Benjamin et al., 1996). Unlike many other isoprenoid-emitting plants, *Eucalyptus* spp. emit both isoprene and monoterpenes, but the emission of isoprene is by far more prevalent (Guenther et al., 1991; Pio et al., 1996; Street et al., 1997; He et al., 2000). Because of these characteristics, *Eucalyptus* has been chosen as the plant species more suitable for modeling purposes (Guenther et al., 1991).

Eucalyptus spp. are native to Australia (Zacharin 1978) but are nowadays among the more widely distributed tree genera around the world. In Mediterranean environments, in particular, they rapidly colonized large areas, particularly in the Iberian peninsula (Pio et al., 1996; Street et al., 1997). Because of their fast growth, *Eucalyptus* spp. trees were planted for commercial purposes, or for limiting land erosion of deforested lands. They became one of the main plant species in urban forests, city parks, and green belts around conurbation. Along the coastal shores of the Mediterranean, *Eucalyptus* spp. were planted because of two other peculiar functions: to break saline wind, and to adsorb saline water and potentially drain soils otherwise unexploitable for agricultural or tourism purposes. Consequently, *Eucalyptus* spp. groves often cope with saline stresses.

Environmental stresses have a strong effect on isoprene emission by plants. Isoprenoid emission is stimulated at elevated temperatures (Loreto and Sharkey, 1990), probably because of the concurrent activation of isoprene synthase (Monson et al., 1992). Water stress seems to have limited effect on isoprene emission even if photosynthesis is severely inhibited. When water stress is relieved, however, isoprene emission can transiently increase 3- to 4-fold over the prestress emission rate (Sharkey and Loreto, 1993).

Salt stress often mimics water stress in two ways. First, when moderate, both stresses limit CO_2 entry by reducing stomatal and mesophyll conductance;

[1] This research was supported in part by the European Union project "Parameterisation of environmental and physiological controls of volatile organic emissions from European forests" (no. ENV4–CT97–0412) and in part by a grant of the University of Molise to S.D.

[2] Present address: Dipartimento di Scienze Animau, Vegetali e deu'Ambiente, Università degli Studi del Molise, via De Sanctis, 86100 Campobasso, Italy.

* Corresponding author; e-mail franci@nserv.icmat.mlib.cnr.it; fax 39–06–9064492.

and second, when severe, they impair carbon metabolism (Delfine et al., 1998, 1999). We investigated the unknown effect of salt stress on isoprene emission of *Eucalyptus globulus*. Because of the wide distribution of *E. globulus* groves in saline soil, if salt stress affects isoprene emission, this environmental stress may have a relevant impact on the isoprene presence in the atmosphere over the Mediterranean countries. In particular, we wanted to test if, as in the case of water stress, recovery from moderate exposure to salt stress could lead to bursts of isoprene emission that are not taken into account by models predicting isoprene emission. We report results indicating that the processes leading to isoprene emission are by far more resistant than photosynthesis to salt stress, and that a secondary source of isoprene, likely to be independent of photosynthesis, is stimulated by salt-stress conditions.

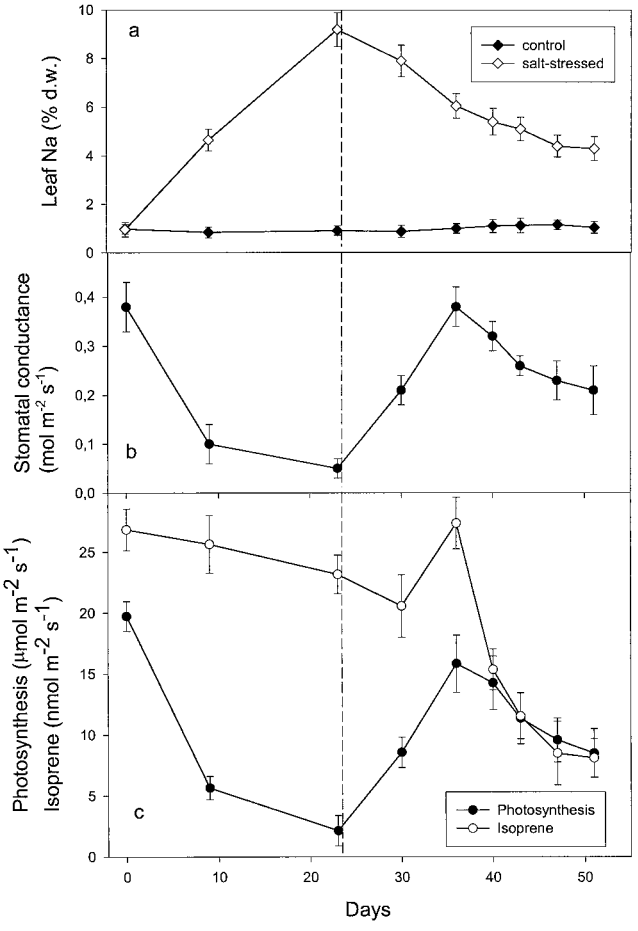

Figure 1. Accumulation of Na (a) and effects of salinity on stomatal conductance (b), photosynthesis, and isoprene emission (c) of *E. globulus* leaves. Means ± SE (n = 5 in a; n = 4 in b and c) are presented. Irrigation with water supplemented with 1% (w/v) NaCl was initiated at d 0. The dashed vertical line indicates the day at which the NaCl treatment was ended and plants were irrigated with salt-free water (recovery). b and c only refer to salt-stressed leaves.

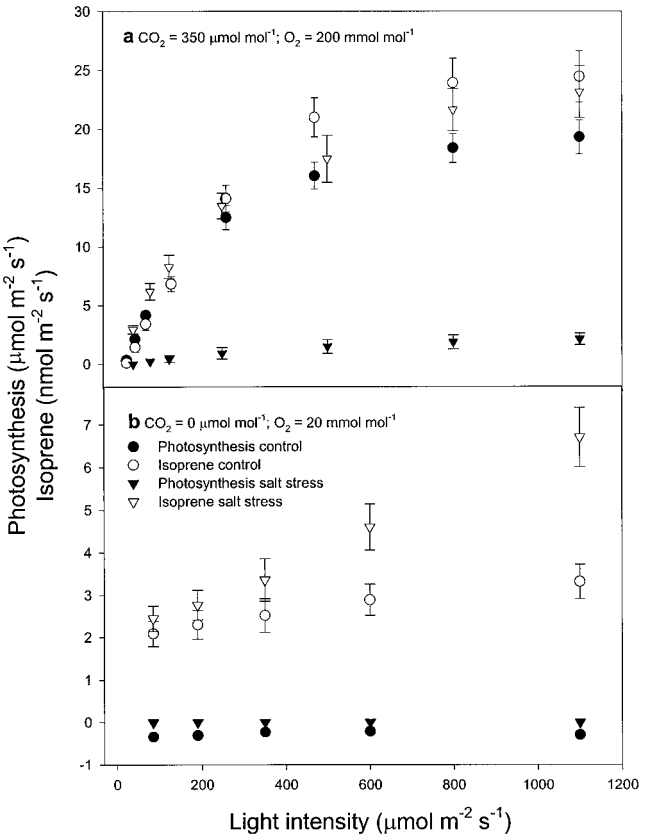

Figure 2. Response of photosynthesis (black symbols) and isoprene emission (white symbols) to light intensity in control (circles) and salt-stressed leaves (triangles) under ambient air conditions (a) and when photosynthesis and photorespiration were inhibited by removing CO_2 and lowering O_2 concentration in the air (b). Means ± SE (n = 4) are shown.

RESULTS

Na accumulated rapidly in the leaves of *E. globulus* and was more than 8% of the leaf dry weight after 3 weeks of NaCl feeding (Fig. 1a). Irrigation with salt-free water decreased the internal concentration of Na but the content was still about 4% of the leaf dry weight after a 1-month recovery.

Leaf stomatal conductance (Fig. 1b) and photosynthesis (Fig. 1c) were inhibited by Na accumulation. After 3 weeks both parameters were very low. Following a 2-week irrigation with salt-free water, leaf stomatal conductance and photosynthesis substantially recovered but these high values were not maintained and slowly dropped until the end of the experiment.

Isoprene emission was not severely affected by Na accumulation (Fig. 1c). We observed a slight reduction of the emission during the stress, which did not promptly recover when the stress was relieved. However, coincident with the maximum recovery of photosynthesis and stomatal conductance, isoprene emission showed a clear peak. The emission then dropped consistent with the reduction of the other physiological parameters.

Photosynthesis of salt-stressed leaves was inhibited at all light intensities with respect to controls (Fig. 2a). Isoprene emission, in contrast, was not inhibited at low-light intensity. At high light the emission of stressed leaves was marginally reduced with respect to the emission of controls. After inhibiting photosynthesis and photorespiration by CO_2 and O_2 removal (Fig. 2b), isoprene emission dropped with respect to that measured in ambient conditions, but it was still measurable. In particular, isoprene emission of salt-stressed leaves was significantly higher than that of controls at light intensities >200 μmol m^{-2} s^{-1}. At the highest light intensity the emission of salt-stressed leaves was twice that of controls.

Photosynthesis was artificially reduced by lowering CO_2 concentration only. At the same intercellular CO_2 concentrations, photosynthesis was by far lower in salt-stressed leaves than in controls, but the compensation point between photosynthesis and photorespiration did not change (Fig. 3). Isoprene emission was not inhibited until intercellular CO_2 concentration was lower than 140 μmol mol^{-1} in controls. In salt-stressed leaves, isoprene emission started to drop significantly only at intercellular CO_2 concentrations lower than 80 μmol mol^{-1}. Both in controls and salt-stressed leaves a significant amount of isoprene was still emitted in CO_2-free air.

Photosynthesis was maximal at a leaf temperature of 25°C in control leaves (Fig. 4a). Temperatures higher than 35°C inhibited photosynthesis. In salt-stressed leaves the rate of photosynthesis was very low at all temperatures. Isoprene emission was stimulated by high temperatures and was maximum at about 40°C but decreased at higher temperatures under non-photosynthetic and non-photorespiratory conditions. As observed in Figure 2b, when photosynthesis and photorespiration were inhibited by CO_2 and O_2 removal, isoprene emission was reduced but still measurable (Fig. 4b). As for the light dependence of this emission, salt-stressed leaves emitted more isoprene than controls at all temperatures. Remarkably, the maximum emission of salt-stressed leaves maintained under CO_2-free and low-O_2 air was not observed at 40°C, as in the respective controls and in all leaves maintained in ambient air, but at 45°C, a temperature at which the emission in ambient conditions was already significantly reduced.

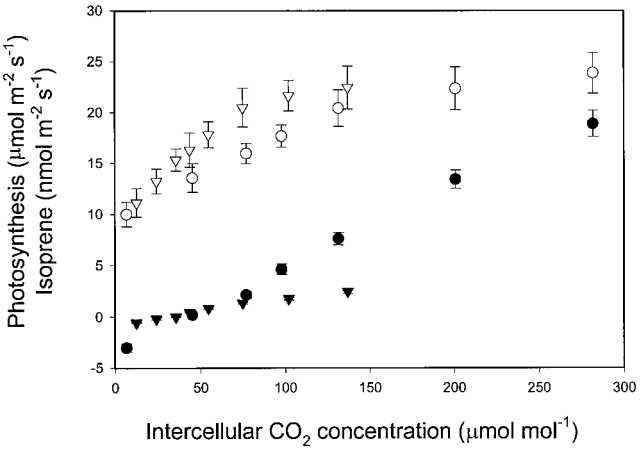

Figure 3. Response of photosynthesis and isoprene emission to intercellular CO_2 concentrations at external CO_2 concentration decreasing from ambient (350 μmol mol^{-1}). Symbol legend as in Figure 2. Means ± SE (n = 4) are shown.

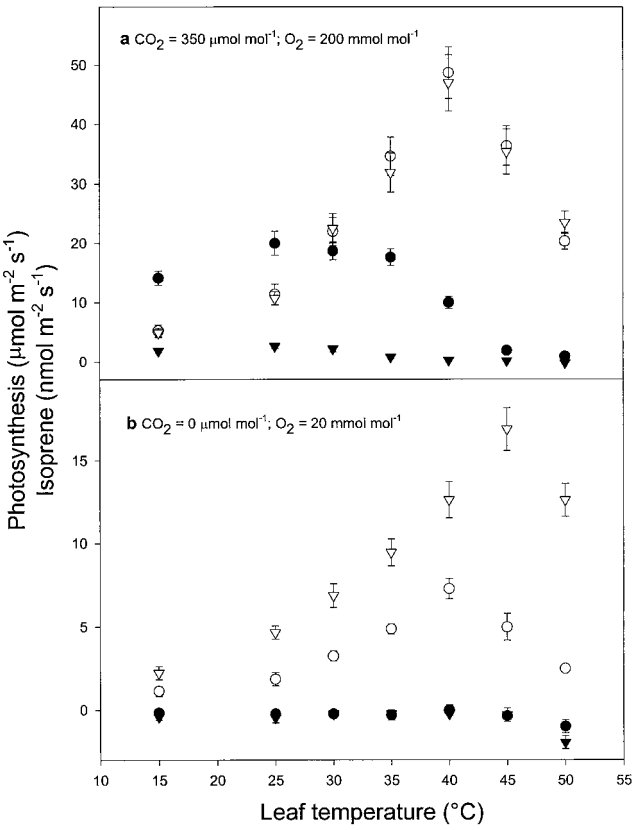

Figure 4. Response of photosynthesis and isoprene emission to leaf temperatures from 15°C to 50°C in control and salt-stressed leaves under ambient air conditions (a) and when photosynthesis and photorespiration were inhibited by removing CO_2 and lowering O_2 concentration in the air (b). Means ± SE (n = 4) are shown. Symbol legend as in Figure 2.

DISCUSSION

Salt stress significantly affected carbon assimilation but did not reduce to a large extent the emission of isoprene by *E. globulus* leaves. The reduction of carbon assimilation is partly caused by the increased resistances to CO_2 diffusion because of stomatal closure (Fig. 1b). However, the slope of the response of photosynthesis to intercellular CO_2 concentration (Fig. 3) is lower in salt-stressed than in control plants, which indicates that carbon metabolism is also directly affected by the stress as often reported (Seemann and Sharkey, 1986; Brugnoli and Björkman, 1992). Delfine et al. (1998) demonstrated that the slope of the photosynthesis to intercellular CO_2 con-

centration ratio does not correctly indicate Rubisco activity if mesophyll resistance is high. They also showed that mesophyll resistance increased during salt-stress episodes and was partially eased during recovery from the stress, and they suggested that diffusion resistance is solely responsible for photosynthesis limitation under moderate salt stress (Delfine et al., 1999). As reported by Delfine et al. (1999), we also found that photosynthesis and stomatal conductance almost completely, though transiently, recovered when salt accumulation was reversed (Fig. 1). Thus the inhibition of photosynthesis in salt-stressed *E. globulus* leaves might only be attributable to diffusion (stomatal and mesophyll) resistances.

Photosynthesis inhibition may inhibit all processes metabolizing photosynthetic carbon. Isoprene is formed from photosynthetic carbon as indicated by the light and CO_2 dependence (Loreto and Sharkey, 1990), and by the fast labeling by ^{13}C (Delwiche and Sharkey, 1993). Isoprenoid synthesis in the chloroplasts was recently found to share a unique pathway involving glyceraldehyde-3-P and pyruvate (Lichtenthaler et al., 1997). Despite isoprene dependence on photosynthetic carbon, isoprene inhibition was very limited in salt-stressed leaves. A low and delayed inhibition of isoprene emission was found in severely water-stressed kudzu leaves (Sharkey and Loreto, 1993). Water-stress recovery resulted in an isoprene emission up to 5 times higher than in controls (Sharkey and Loreto, 1993). We also found a peak of isoprene emission in *E. globulus* leaves 2 weeks after rewatering with salt-free water. Our results therefore confirm that environmental stresses do not significantly affect, and that recovery from stresses can temporarily stimulate, the emission of isoprene. After the peak, isoprene emission dropped in leaves recovering from salt stress. This drop occurred in concert with the drop of photosynthesis and stomatal conductance; we attribute these simultaneous effects to leaf aging. It is known that salt-stress accelerates aging of leaves (Delfine et al., 1999) and that isoprene emission is strongly dependent on leaf age (Sharkey and Loreto, 1993).

Both water and salt stress primarily affect stomatal conductance. The isoprene burst in recovering leaves could therefore reflect the release of isoprene synthesized but not emitted because of stomatal closure. Isoprene is emitted through stomata (Fall and Monson, 1992). However, there are numerous reports showing that stomata are not able to regulate isoprene emission even if they are tightly closed (Loreto and Sharkey, 1990; Sharkey, 1991; Fall and Monson, 1992). Isoprene builds-up in the leaves compensating for the increased stomatal resistances to its diffusion in air (Fall and Monson, 1992). We found that isoprene emission does not drop until a low-intercellular CO_2 concentration is reached (Fig. 3). This effect was even exacerbated in salt-stressed leaves that maintained a similar emission rate at intercellular CO_2 concentration higher than 80 μmol mol^{-1}.

Photosynthesis inhibition by salt stress was clear at different light intensities and leaf temperatures. However, we found no significant differences between isoprene emission in controls and salt-stressed leaves at reduced light intensities (Fig. 2a), at very low-intercellular CO_2 concentration (Fig. 3), and at increasing temperatures (Fig. 4a). On the contrary, we found that isoprene emission was actually increased in salt-stressed leaves with respect to controls when the light dependence and the temperature dependence were tested under non-photosynthetic and non-photorespiratory conditions (Figs. 2b and 4b). It is known that isoprene emission is severely reduced but not totally inhibited by simultaneous removal of CO_2 and O_2 (Loreto and Sharkey, 1990). This indicates that additional, non-photosynthetic sources of carbon can be used to form isoprene. The isoprene-like synthesis of monoterpenes in *Quercus ilex* leaves was not totally inhibited in the dark and exposure to darkness of leaves after a complete labeling of the emission resulted in a increasing amount of unlabeled monoterpenes emitted by and contained in the leaves (Loreto et al., 2000). These findings also indicate the presence of alternative routes of isoprenoid synthesis. Our results indicate that these routes are activated by salt stress and that perhaps a similar effect may be exerted by a range of environmental and biotic stresses. In fact, this would help explain why isoprene emission is sustained while photosynthesis is inhibited both in water- and salt-stressed leaves. The activation of photosynthesis-independent routes of isoprene synthesis makes it unclear if isoprene emission can be modeled on the basis of the leaf photosynthetic properties (Niinemets et al., 1999) under stress conditions.

Perhaps the most remarkable difference between the emission of control and salt-stressed leaves maintained under non-photosynthetic and non-photorespiratory conditions was observed at very high temperatures. Both controls and salt-stressed leaves showed the typical temperature dependence of isoprene emission (Monson et al., 1992; Sharkey and Loreto, 1993; Harley et al., 1996), which mirrors the activation of isoprene synthase (Monson et al., 1992). However, isoprene emission was maximal at 40°C in control leaves, but at 45°C in salt-stressed leaves. These different maxima correspond to the top activities of the stromal and thylakoid-bound isoprene synthases (Fall and Wildermuth, 1998). The observed temperature dependency might therefore reflect the increasing contribution of the thylakoid-bound isoprene synthase to isoprene emission in salt-stressed leaves. However, the high variability of temperature optima for isoprene synthases (Fall and Wildermuth, 1998) does not justify more than speculations. Certainly, the enzyme catalyzing isoprene synthesis in

salt-stressed leaves resists temperature denaturation or down-regulation more effectively than in controls. This may have an ecological explanation if isoprene becomes more important in protecting membranes against thermal denaturation (Sharkey and Singsaas, 1995) in salt-stressed leaves.

In conclusion, our experiments show that isoprene synthesis is resistant to salt stress and, confirming previous experiments on water-stressed leaves, suggest that isoprene emission is rather unaffected by the stress. Isoprene emission can even be transiently stimulated by salt (and water) stress, perhaps because of the activation of alternative routes of isoprene synthesis under stress conditions. The regular and even enhanced investment of carbon into isoprene formation under stress conditions encourages the idea that isoprene is involved in resistance mechanisms and should be considered when modeling the emission from Mediterranean vegetation recurrently exposed to salt stress.

MATERIALS AND METHODS

Three-year-old plants of *Eucalyptus globulus* were grown in open air during summer 1999 at the Consiglio Nazionale delle Ricerche, Istituto di Biochimica ed Ecofisiologia Vegetali experimental field, near Rome (42° N), under the environmental conditions typical of summer in the Mediterranean area (maximal temperature >35°, absence of rain). Twenty plants were grown in 50-L pots filled with sandy and fertilized soil and were irrigated daily to restore evapotranspiration losses and avoid water stress. At the beginning of August one-half of the plants were irrigated with water supplemented with 1% (w/v) NaCl. After 3 weeks of salt accumulation, corresponding to a very low photosynthesis of salt-stressed plants, the NaCl treatment was suspended and the effect of salt dilution by irrigation with salt-free water was followed for 1 month.

Na accumulated in the leaves was extracted from 150 mg of dry leaf mass with the procedure described by Delfine et al. (1999) and was measured by atomic emission spectrometry (ICP Plasma 40, Perkin-Elmer, Foster City, CA).

Photosynthesis and stomatal conductance were measured using the gas-exchange system described by Delfine et al. (1998). The leaf portion (5 cm^2) clamped in the gas-exchange cuvette was maintained at temperatures between 15°C and 50°C and at light intensities between 0 and 1,100 μmol photons m^{-2} s^{-1}. The cuvette was flushed with a 1 L of synthetic air made by mixing N_2, O_2, and CO_2, and did not contain ozone, isoprenoids, and other contaminants. The CO_2 dependence of the emission was checked at CO_2 concentrations between zero and ambient (350 μmol mol^{-1}). A set of measurements was done in CO_2-free air and low O_2 (20 mmol mol^{-1}) to inhibit both photosynthesis and photorespiration. Measurements of gas-exchange and isoprene emission during the salt-stress treatment and the following recovery were done at a leaf temperature of 30°C and under a light intensity of 1,000 μmol m^{-2} s^{-1}. The relative humidity of the air was maintained at 40% to 50% during all measurements. The leaf was maintained at least 30 min in each experimental condition to reach steady photosynthesis and isoprene emission before measurements.

Isoprene emission was measured by diverting a part of the air exiting the cuvette (40 mL) into a gas-chromatograph (Syntech GC855 series 600, Syntech, Groningen, The Netherlands). The air was pumped through a Graphite-Tenax (60–80 mesh, 8-cm) trap, desorbed at 240°C, transferred to a 13-m capillary column (i.d. 0.53 mm, packed with 95% [w/v] dimethylpolysiloxane, 5% [w/v] diphenylpolysiloxane) under a flow of pure N_2, and the isoprene present was detected after 198 s by photoionization at 10.6 eV. The gas chromatograph was calibrated with several concentrations of gaseous isoprene and monoterpenes and showed a linear response even at the highest monoterpene concentrations. Additional comparisons were done with simultaneous measurements of isoprene and monoterpenes trapped in carbon cartridges and analyzed by gas chromatography-mass spectrometry (Loreto et al., 1996).

All measurements were made on the last fully expanded leaf of tree branches. Na accumulation was measured in five leaf discs of different plants for each data point. Gas-exchange and isoprene emission measurements were repeated on four different leaves of different plants for each data point. Means and SE of the measurements are shown. Measurements of photosynthesis, isoprene emission, and Na accumulation were repeated approximately every week during the stress and the following recovery as shown in Figure 1. Measurements of the light, CO_2, and temperature dependency (Figs. 2–4) were made in controls and salt-stressed leaves when Na accumulation was maximum (18–22 d after starting the treatment).

ACKNOWLEDGMENTS

We thank Michela Mannozzi, Pamela Nascetti, and Domenico Tricoli for helping with the gas-exchange and gas-chromatographic measurements. Dr. Alberto Palmieri is gratefully acknowledged for the use of the atomic emission spectrometer.

Received February 23, 2000; accepted May 4, 2000.

LITERATURE CITED

Benjamin MT, Sudol M, Bloch L, Winer AM (1996) Low-emitting urban forests: a taxonomic methodology for assigning isoprene and monoterpenes emission rates. Atmos Environ **30:** 1437–1452

Brugnoli E, Björkman O (1992) Growth of cotton under continuous salinity stress: influence on allocation pattern, stomatal and non-stomatal components of photosynthesis and dissipation of excess light energy. Planta **187:** 335–347

Delfine S, Alvino A, Villani MC, Loreto F (1999) Restrictions to CO_2 conductance and photosynthesis in spinach leaves recovering from salt stress. Plant Physiol **119:** 1101–1106

Delfine S, Alvino A, Zacchini M, Loreto F (1998) Consequences of salt stress on diffusive conductances, Rubisco characteristics and anatomy of spinach leaves. Aust J Plant Physiol **25:** 395–402

Delwiche CF, Sharkey TD (1993) Rapid appearance of ^{13}C in biogenic isoprene when $^{13}CO_2$ is fed to intact leaves. Plant Cell Environ **16:** 587–591

Fall R, Monson RK (1992) Isoprene emission rate in relation to stomatal distribution and stomatal conductance. Plant Physiol **100:** 987–992

Fall R, Wildermuth MC (1998) Isoprene synthase: from biochemical mechanism to emission algorithm. J Geophys Res **103:** 25599–25609

Guenther AB, Hewitt CN, Erickson D, Fall R, Geron C, Graedel T, Harley PC, Klinger L, Lerdau M, McKay WA, Pierce T, Scholes B, Steinbrecher R, Tallamraju R, Taylor J, Zimmermann P (1995) A global model of natural volatile organic compound emissions. J Geophys Res **100:** 8873–8892

Guenther AB, Monson RK, Fall R (1991) Isoprene and monoterpene emission rate variability: observations with eucalyptus and emission rate algorithm development. J Geophys Res **96:** 10799–10808

Harley P, Guenther A, Zimmerman P (1996) Effects of light, temperature and canopy position on net photosynthesis and isoprene emission from sweetgum (*Liquidambar styraciflua*) leaves. Tree Physiol **16:** 25–32

He C, Murray F, Lyons T (2000) Monoterpene and isoprene emissions from 15 *Eucalyptus* species in Australia. Atmos Environ **34:** 645–655

Jones CA, Rasmussen RA (1975) Production of isoprene by leaf tissue. Plant Physiol **55:** 982–987

Lichtenthaler HK, Schwender J, Disch A, Rohmer M (1997) Biosynthesis of isoprenoids in higher plant chloroplasts proceeds via a mevalonate-independent pathway. FEBS Lett **400:** 271–274

Loreto F, Ciccioli P, Brancaleoni E, Frattoni M, Delfine S (2000) Incomplete ^{13}C labeling of alpha-pinene content in *Quercus ilex* leaves and appearance of unlabeled C in alpha-pinene emission in the dark. Plant Cell Environ **23:** 229–234

Loreto F, Ciccioli P, Cecinato A, Brancaleoni E, Frattoni M, Tricoli D (1996) Influence of environmental factors and air composition on the emission of α-pinene from *Quercus ilex* leaves. Plant Physiol **110:** 267–275

Loreto F, Sharkey TD (1990) A gas exchange study of photosynthesis and isoprene emission in red oak (*Quercus rubra* L.). Planta **182:** 523–531

Monson RK, Fall R (1989) Isoprene emission from aspen leaves. Plant Physiol **90:** 267–274

Monson RK, Harley PC, Litvak ME, Wildermuth M, Guenther AB, Zimmerman PR, Fall R (1994) Environmental and developmental controls over the seasonal pattern of isoprene emission from aspen leaves. Oecologia **99:** 260–270

Monson RK, Jaeger CH, Adams WW, Driggers EM, Silver GM, Fall R (1992) Relationships among isoprene emission rate, photosynthesis, and isoprene synthase activity as influenced by temperature. Plant Physiol **98:** 1175–1180

Niinemets U, Tenhunen JD, Harley PC, Steinbrecher R (1999) A model of isoprene emission based on energetic requirements for isoprene synthesis and leaf photosynthetic properties for *Liquidambar* and *Quercus*. Plant Cell Environ **22:** 1319–1335

Pio CA, Nunes TV, Valente AR (1996) Biogenic hydrocarbon emissions from vegetation in a southern european environment. *In* PM Borrel, P Borrell, T Cvitas, K Kelly, W Seiler, eds, Proceedings of the EUROTRAC Symposium 96. Computational Mechanics, Southampton, UK, pp 35–43

Seemann JR, Sharkey TD (1986) Salinity and nitrogen effects on photosynthesis, ribulose-1,5-bisphosphate carboxylase and metabolite pool size in *Phaseolus vulgaris* L. Plant Physiol **82:** 555–560

Sharkey TD (1991) Stomatal control of trace gas emissions. *In* TD Sharkey, EA Holland, HA Mooney, eds, Environmental and Metabolic Controls of Trace-Gas Emission by Plants. Academic Press, New York, pp 335–339

Sharkey TD, Loreto F (1993) Water stress, temperature, and light effects on the capacity for isoprene emission and photosynthesis of kudzu leaves. Oecologia **95:** 328–333

Sharkey TD, Loreto F, Delwiche CF (1991) The biochemistry of isoprene emission from leaves during photosynthesis. *In* TD Sharkey, EA Holland, HA Mooney, eds, Environmental and Metabolic Controls of Trace-Gas Emission by Plants. Academic Press, New York, pp 153–184

Sharkey TD, Singsaas EL (1995) Why plant emits isoprene. Nature **374:** 769

Street RA, Hewitt CN, Mennicken S (1997) Isoprene and monoterpene emissions from a *Eucalyptus* plantation in Portugal. J Geophys Res **102:** 15875–15887

Zacharin RF (1978) Emigrant *Eucalyptus*. Melbourne University Press, Melbourne, pp 4–11