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Effects of different doses of nitrogen treatments on isoprene emission from *Ficus glomerata*

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Abstract: In the present investigation on the effect of nitrogen treatments on emission of isoprene from *Ficus glomerata* has been evaluated. Four sets of plants were treated with following four doses of nitrogen in the form of ammonium nitrate solution (i) 10 mM (ii) 50 mM (iii) 100 mM (iv) 200 (mM) and control set was designed without any treatment. Nitrogen treated as well as control plants were sampled for isoprene emission using a dynamic flow through enclosure chamber technique and samples were analysed with the help of GC-FID. Isoprene emissions from control, 10 mM, 50 mM, 100 mM, and 200 mM nitrogen treated plants were found to be 27.5 ± 4 $\mu gg^{-1}h^{-1}$, 56 ± 6 $\mu gg^{-1}h^{-1}$, 91 ± 11 $\mu gg^{-1}h^{-1}$, 101 ± 10 $\mu gg^{-1}h^{-1}$, and 15 ± 4 $\mu gg^{-1}h^{-1}$ respectively. Lowest isoprene emission (15 ± 4 $\mu gg^{-1}h^{-1}$) was noticed in plants treated with 200 mM nitrogen. Isoprene emissions were found to increase exponentially in plants treated with nitrogen up to 100 mM. Nevertheless, plants treated with 200 mM nitrogen exhibited decrease in emission by 46 per cent, probably on account of nitrite toxicity and reduction in soil pH at high nitrogen dose.

Keywords: Isoprene, Nitrogen, Emission, Plants, Enclosure chamber

INTRODUCTION

Isoprene (2-methyl 1, 3-butadiene) is most dominant and highly reactive volatile organic compound emitted from leaves of many plant species, which is estimated to contribute 44 per cent to the global biogenic VOC budget of 1150 Tg Cyr^{-1} (Guenther *et al.,* 1995). Its annual global emission is estimated at 500 Tg C $year^{-1}$ from vegetation to the atmosphere (Guenther *et al.,* 1995). The emission of isoprene has a profound impact on the state and dynamics of atmospheric chemistry (Fehsenfield *et al.,* 1992). In the atmosphere, isoprene rapidly reacts with nitrogen oxides (NOx), hydroxyl radicals, NO_3, and ozone and produces a wide range of compounds including carbonyls, organic acids, ozone, CO and aerosols (Zimmerman *et al.,* 1978; Jacob and Wofsy, 1988 and; Chameides *et al.,* 1992). It has been reported that isoprene emission is species specific varying as much as four order of magnitude depending upon plant species (Benjamin *et al.,* 1996). Various studies have shown that isoprene emission from vegetation is influenced by temperature (Tingey *et al.,* 1979), solar radiation (Harley *et al.,* 1996), season (Singh *et al.,* 2008) and soil nitrogen level (Lerdau *et al.,* 1995). Little information is currently available on soil nitrogen levels effects on isoprene emission. Common Indian plant species have been examined for isoprene emission (Varshney and Singh, 2003; Singh and Varshney, 2006; Singh *et al.,* 2007 and Singh *et al.,* 2008). However, studies are altogether lacking from the Indian sub continent on soil nitrogen levels effects on isoprene emission. This study reports for the first time effects on soil nitrogen levels on isoprene emission from *Ficus glomerata* plant species.

MATERIALS AND METHODS

Ficus glomerata plant one inch long saplings were planted in pots containing ordinary loam soil without any organic or inorganic matter supplement. Five sets of these plants were maintained up to a height of two and half feet and watered regularly. Each set comprised of three plants. Out of five sets of plants, four sets of plants were treated with nitrogen, whereas, fifth set of plants were maintained as control (without any treatment). Nitrogen was given to plants in the form of ammonium nitrate solution. Fifteen days old saplings were treated with following four doses of nitrogen in two installments (i) 10 mM (ii) 50 mM (iii) 100 mM (iv) 200 mM solution of ammonium nitrate. Eight months old controls as well as nitrogen treated plants were twice sampled for isoprene emission measurements. The study was carried out from February 2004 to October 2005. Temperature and photo synthetically active radiation (PAR) were measured both inside and outside the chamber with a thermometer and Li Cor Quantum sensor (Model LI-185) respectively.

Isoprene emissions from plants were sampled using a dynamic flow through enclosure system as employed

previously by Winer *et al.* (1989). The enclosure chamber was constructed from 0.25 mM transparent polycarbonate sheet measuring approximately 38 cm × 39 cm × 46 cm**.** The enclosure chamber was equipped with a fan and inlet and outlet ports suitable for introduction of matrix air and withdrawal of analytical samples respectively. The enclosure was carefully fitted around the top of the plant sapling in order to minimise any effect from rough handling. Air was passed through the enclosure chamber at a rate of 20 L min^{-1} and this flow was maintained for 20 minutes prior to sampling. Samplings were carried out for 10 minutes as described by Winer *et al.* (1989) at a rate of 0.10 L min^{-1} from enclosure onto Tenax TA (200 mg)/carbosieve (100 mg) II solid adsorbent (Supelco Inc. Bellefonte, PA). The packed Tenax TA/carbosieve tubes were preconditioned by heating at 300 ^{0}C for 48 hours with a continuous purge of nitrogen. The isoprene trapping efficiency of tube was 98 per cent as checked with isoprene standard**.** In order to eliminate the effect of isoprene present in ambient air and adsorbed on the surfaces of the chamber, a blank with no sapling branch in the enclosure chamber was sampled immediately before each measurement. The values of blank samples were subtracted from the measurement value. After sampling Tenax tubes were sealed with Teflon ferrules and stored at 4 ^{0}C and the samples were analysed within 30 minutes. After completion of the emission flux measurements, the branch enclosed in the chamber, was harvested, and the leaves were stripped off the stems and then dried in an oven at 70 ^{0}C to a constant weight.

Quantification of the isoprene was carried out using a Nucon gas chromatograph (Model 5765, Nucon Engineers, Okhla, New Delhi) to a fused silica capillary column (length: 30 m, id: 0.53 mM, bonded phase BP-90 I, Alltech Associates, Dearfield, IL, USA) attached to the flame ionization detector (FID) was used for isoprene determination. Compounds were desorbed at 280 ^{0}C for 8 minutes onto a Tenax TA/carbosieve by a thermal desorber injection system (Nucon Engineers, Okhla, New Delhi) attached with the GC. The initial oven temperature was maintained at 40 ^{0}C for 5 minutes, and then increased to 180 ^{0}C at a rate of 5 ^{0}C min^{-1} for 5 minutes. Thereafter temperature increased at a rate of 15 ^{0}C up to 250 ^{0}C and was maintained for 10 minutes. N_2 was used as carrier gas and the flow rate was maintained at 8 ml min^{-1}, the injection temperature was 230 ^{0}C and detector temperature was 250 ^{0}C. Isoprene in the samples was determined with the help of a standard calibration plot prepared from the liquid chemical standard of isoprene obtained from Fluka/Sigma-Aldrich, USA. Gas phase liquid chemical standard of isoprene was prepared by serial dilution in 500 ml round flasks fitted with screw cap syringe sampling ports. A weekly calibration was performed for isoprene. Four different concentrations of isoprene i.e. 10, 50, 100 and 200 ppb (in 100 cm^3 of air) were drawn in to a 100 cm^3 gas tight syringe (Hamilton and co.) and injected in to the Tenax end of the Tenax TA/ carbosieve tubes and tubes were placed directly into the injection port and desorbed with the Tenax end directly above the column. To prevent any loss of the standards, less than 4 seconds elapsed between placing the sample tube in the injection port and placement of the cover and less than 40 seconds usually elapsed between the placement of the insert into the injection port and the start of the run. Response factors were generated by dividing the standard concentration by the peak area for isoprene at that concentration and multiplying by the volume of standard taken in liter. Response factors were used for the calculation of the isoprene concentrations from the observed peak areas. The precision and accuracy of the GC/FID system were about 4 per cent as determined by repeated measurements of the standard gas. The detection limit for isoprene was 0.01 ng on column, corresponding to 2 pptv isoprene. The isoprene emission rate, MR (μ g g^{-1} h^{-1}) for individual plant species was calculated as: $MR = V (C_i - C_0) W^{-1}t^{-1}$

Where (C_i - C_0) is the difference in the concentration of isoprene for a given time interval, t is the sampling time (h), W is dry weight of leaves within the enclosure (g) and V is the volume of the enclosure system (m^{-3}).

Measured isoprene emission rates were normalised to PAR and temperature of 1000 μ mol m^{-2} s^{-1} and 30 ^{0}C, respectively, using the algorithm proposed by Guenther *et al.* (1993) and subsequently modified by Guenther (1997). Normalisation of emission rates facilitates isoprene emission factor estimation and comparison of isoprene emission rates of this study with previous studies and comparison between plant species.

In this algorithm, isoprene emission rates are described as: $I = MR \times C_L \times C_T$ (i)

I is emission rate [μ g g^{-1} h^{-1}] at current leaf temperature T (K) and PAR intensity L (μ mol m^{-2} s^{-1}). MR is a base emission rate at standard temperature T_S (303 K) and PAR intensity (1000 μ mol m^{-2} s^{-1}).

The two variables C_L and C_T are respectively light and

Table 1. Effect of different doses of nitrogen on isoprene emission from *Ficus glomerata.*

S. No.	N_2 treatment dose (mM)	N.I.E.R. (μg $g^{-1}h^{-1}$)	Increase/Decrease over control (per cent)
1	10	56 ± 6	57.0
2	50	91 ± 11	69.78
3	100	101 ± 10	72.77
4	200	15 ± 4	- 46
5	Control	27.5 ± 4	-

N.I.E.R.: Normalised isoprene emission rate

temperature coefficients derived from experimental measurements on *Eucalyptus*, sweet gum, aspen, and velvet bean and are defined by

$C_L = \alpha \times C_{L1} \times L \times [1+ \alpha^2 + L]^{-1/2}$ (ii)

Where L was the PAR (μ mol m^{-2} s^{-1}), C_{L1} was an empirical coefficient (1.067) and a was an empirical coefficient (0.0027),

C_T is calculated as follows:

$C_T = \exp. \{C_{T1} (T- Ts) (R \times Ts \times T)^{-1}\} / 0.961 + \exp.\{ C_{T2} \times (T- Tm) (R \times Ts \times T)^{-1} \}$ (iii)

Where, T was the leaf temperature in Kelvin, R was a gas constant (8.314 J K^{-1} mol^{-1}), Ts was the normalising temperature in degree Kelvin; Tm was an empirical coefficient (315 K); C_{T1} a was an empirical coefficient (95, 000 J mol^{-1}), and C_{T2} was an empirical coefficient (230, 000 J mol^{-1})

RESULTS AND DISCUSSION

Isoprene emissions from control, 10 mM, 50 mM, 100 mM, and 200 mM nitrogen treated plants were found to be 27.5 ± 4 μg$g^{-1}h^{-1}$, 56 ± 6 μg$g^{-1}h^{-1}$, 91 ± 11 μg$g^{-1}h^{-1}$, 101 ± 10 μg$g^{-1}h^{-1}$, and 15 ± 4 μg$g^{-1}h^{-1}$ respectively (Table 1). As compared to nitrogen treated plants (except 200 mM treated plants), relatively low isoprene emissions (27.5 ± 4 μg$g^{-1}h^{-1}$) were observed from control plants, grown on ordinary loam soil without any organic or inorganic supplement. It may be due to nitrogen availability link with carbon required for growth; low nitrogen plant would have reduced carbon demand because of nitrogen limitations. This would obviate the need to mobalise carbon that is likely to release as VOC. A moderate correlation ($r^2 \leq 0.048$, $p < 0.05$) between plants nitrogen treatment and isoprene emissions were noticed (Fig. 1). Isoprene emissions were found to increase exponentially in plants treated with nitrogen up to 100 mM (Fig. 1). Increase in isoprene emission in nitrogen treated plants has also been reported by Lerdau *et al.* (1995). Increase in isoprene emission in nitrogen treated plants could be on account of increased availability of nitrogen to the plants resulting optimum expression of isoprene synthase enzyme activity available for the synthesis of isoprene. It is also possible that isoprene emission increased on account of increase in photosynthesis rate due to nitrogen availability (Lerdau and Throop, 2000; Lerdau *et al.*, 1995). Previous studies have also shown that nitrogen treatments significantly influence photosynthesis (Reich and Walters, 1994; Reich *et al.*, 1994). Studies carried out on nitrogen application effects on isoprene emissions have reported positive correlations between isoprene emission rate and nitrogen availability (Harley *et al.*, 1994; Monson *et al.*, 1994; Lerdau *et al.*, 1995, Funk, 2002). Plants treated with 10 mM nitrogen exhibited 52.3 per cent increase in isoprene emission over control. Whereas, isoprene emission increased by 69.78 per cent and 72.77 per cent in plants treated with 50 mM and 100 mM nitrogen respectively.

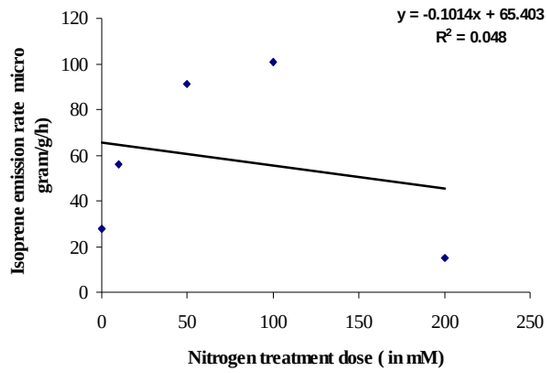

Fig. 1. *Showing correlation between nitrogen treatment and isoprene emissions in the plant Ficus glomerata.*

Maximum rate of isoprene emission increase over control plants were found in plants treated with 10 mM nitrogen (Table 1). However, diminishing return in emission was observed in plants treated with 50 mM and 100 mM of nitrogen as compared to 10 mM nitrogen treated plants. This is possibly due to partial fulfillment of nitrogen requirement of the plants at relatively low dose. Plants treated with 200 mM nitrogen exhibited decrease in emission by 46 per cent (Table 1). This may be due to increase in soil nitrite levels and reduction in soil pH at considerably high nitrogen dose. It has been reported that high concentration of nitrite causes chlorosis and reduces soil pH (Phipps and Chornforth, 1970). Isoprene synthase enzyme activity in plants has a high pH optimum (Sharkey and Yeh, 2001). Therefore, reduction in soil PH could affect isoprene synthesis and emission. Besides, low soil pH increases availability of toxic metals such as aluminum, manganese in the soil (Dong *et al.*, 1995), which may leads to increase in concentration of both metals in the plant tissues. High concentration of both metals damage photosynthetic apparatus (Sharkey and Yeh, 2001), which in turn affect isoprene emission. Previous study has also reported decrease in isoprene emission in Doglas–Fir (*Pseudotsuga menziesii*) plants treated with higher nitrogen concentrations (Lerdau *et al.*, 1995; Funk, 2002). Nevertheless, mechanism of effects of soil nitrogen on isoprene emission is not fully understood and further studies are required to ascertain exact mechanism of nitrogen treatment effects on isoprene emission.

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