Phytochemistry, 1973, Vol. 12, pp. 15 to 19. Pergamon Press. Printed in England.

EMISSION ISOPRENE FROM LEAF DISCS OF *HAMAMELIS*

R. A. RASMUSSEN* and C. A. JONES†

Air Pollution Research Section, College of Engineering Research Division, Washington State University, Pullman, WA 99163, U.S.A.

(*Revised Received* 29 *March* 1972. *Accepted* 23 *June* 1972)

Key Word Index—*Hamamelis jelena*; Hamamelidaceae; isoprene production; light-dependence; DCMU and DNP inhibition.

Abstract—Isoprene has been reported and identified as a light dependent natural plant emission. GLC techniques are used to measure isoprene emission from leaf discs. The rate of emission as a function of temperature and light quality is reported. Normal isoprene emission rates for *Hamamelis jelena* leaf discs were between 280 and 790 ng cm^{-2} hr^{-1} at 11 000 lx. A detailed action spectrum showing isoprene emission from 420 to 700 nm is presented. Isoprene emission rates are shown to respond within 1 min to the initiation and cessation of illumination. Both 2,4-dinitrophenol (10^{-3} M) and dichlorodimethylphenylurea (10^{-4} M) substantially reduce isoprene emission.

INTRODUCTION

THE DISCOVERY of isoprene (CH_2=C(Me)–CH=CH_2) as a volatile plant product was made independently by Sanadze[1] and Rasmussen[2] in 1961. Subsequent work has conclusively proved that isoprene (2-methyl-1,3-butadiene) is a natural plant product.[3,4] The effects of light intensity, light quality, temperature, O_2 and CO_2 concentration, and the inhibitor NO_3^- on the production of isoprene and limited ^{14}C studies have been investigated by Sanadze.[5–8]

Upon initiating research into the physiology of isoprene production, it was apparent that several aspects of Sanadze's work needed clarification; his techniques precluded rapid sequential sampling of the isoprene content within his chambers, his action spectrum was inadequate, and no standard deviations were included in his measurements. In view of these limitations in the earlier work, isoprene emission from leaf discs of *Hamamelis jelena* was investigated in more detail.

* Associate Plant Physiologist.
† Graduate Student in Botany.

[1] G. A. SANADZE and G. M. DOLIDZE, *Soobshch. Akad. Nauk Gruz. SSR.* **27**, 747 (1961).
[2] R. A. RASMUSSEN, *Progress Report*, Gas Chromatography Laboratory, Monsanto, Co., St. Louis, Missouri, (Dec., 1961).
[3] R. A. RASMUSSEN, *Environ. Sci. Tech.* **4**, 667 (1970).
[4] R. A. RASMUSSEN and F. W. WENT, *Proc. Nat. Acad. Sci.* **53**, 215 (1965).
[5] G. A. SANADZE, *Fiziol. Rast.* **11**, 49 (1964).
[6] G. A. SANADZE and A. N. KALANDADZE, *Fiziol. Rast.* **13**, 458 (1966).
[7] G. A. SANADZE and A. N. KALANDADZE, *Dokl. Akad. Nauk SSSR* **168**, 227 (1966).
[8] G. A. SANADZE, *Fiziol. Rast.* **13**, 753 (1966).

RESULTS AND DISCUSSION

The amount of isoprene typically produced by dark-adapted leaf discs during three alternate periods of light and dark is shown in Fig. 1. The results show a slightly lower rate of isoprene production during the initial 10 min light period than during subsequent light periods. In addition, a small amount of isoprene is emitted in the dark.

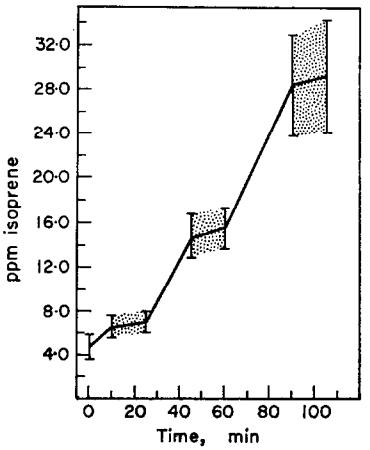

FIG. 1. ISOPRENE PRODUCTION IN LIGHT AND IN DARK.
Light periods of 10, 20 and 30 min are alternated with 15 min of darkness. $N = 8$ leaf discs; bars represent 1 s.d. Headspace volume is corrected to 7 ml.

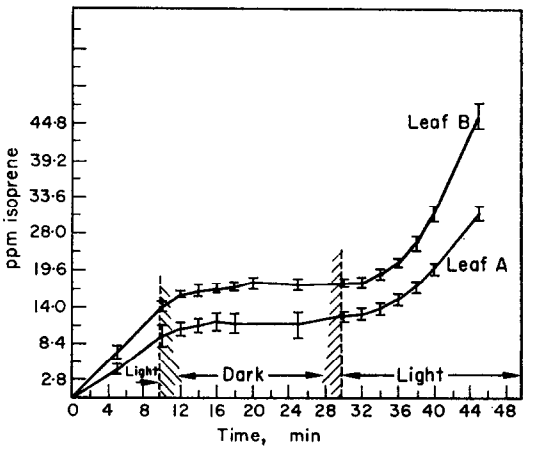

FIG. 2. TURN-OFF AND TURN-ON TIME FOR ISOPRENE PRODUCTION.
Four discs from each of 2 leaves were sampled sequentially. Data is corrected to 10 ml headspace. Bars represent 1 s.d.

Clarification of the turn-on/turn-off behavior in the light and dark was obtained (Fig. 2). The leaf discs were enclosed in the study chambers in the light. Consequently, no turn-on behavior was noted during the initial 10 min. When the leaf discs were placed in the dark. isoprene emission ceased. Distinct inflections in the curves can be seen within 2 min. The rate of isoprene emission returned to normal within 10 min of re-illumination. The gradual inflections in the two curves can be interpreted to represent the finite amount of time it takes for isoprene to diffuse out of the leaf tissues after the synthetic mechanism is turned on by light. Stomatal correlations are improbable as CO_2 accumulates at a constant rate in the chambers in the dark, and the response of isoprene emission to illumination is almost immediate after a prolonged dark period when stomatal apertures would be closed. Figure 2 also shows the greater inter-leaf variation than intra-leaf variation which has occurred consistently throughout our investigations.

Temperature greatly influences the rate of isoprene emission. Sanadze[7] reported a Q_{10} between 25 and 35° of 2·8–3·3. The response of isoprene production to temperature for four different tissues (*Quercus*, *Eucalyptus*, and old and young *Hamamelis* leaves) is shown in Fig. 3 and Table 1. The Q_{10} values 3·1–3·6 and the high temperature (40°) of maximal production of isoprene are consistent with Sanadze's[7] work on *Populus nigra*. These results indicate that our tissues are physiologically comparable to the whole excised leaves which Sanadze used. Also the heat-resistant nature of the mechanism for isoprene production is apparent in the maximal emission rate obtained at 40°. However, even at 15° the rates of

emission represent significant quantities of metabolic product escaping from the leaf. It should be noted that at 40°, 4·4 ppm isoprene ml^{-1} min^{-1} cm^{-2} are emitted from young *Hamamelis* leaves, but less than 0·001 ppm ethylene ml^{-1} min^{-1} cm^{-2} are emitted by the same tissue.

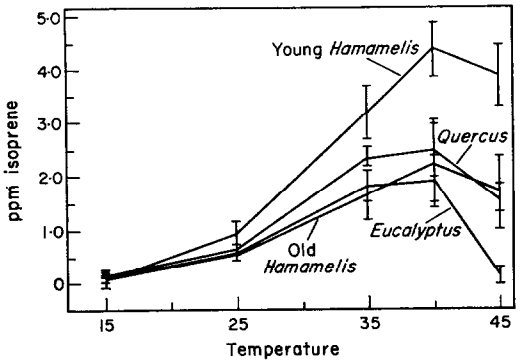

FIG. 3. RESPONSE OF ISOPRENE PRODUCTION TO TEMPERATURE.

$N = 9$ leaf discs placed on water of each temperature, allowed to equilibrate for 15 min, then enclosed. Isoprene concentration was measured after 20 min and is expressed in ppm emitted into 1 ml headspace in 1 min by 1 cm^2 tissue. Bars represent 1 s.d.

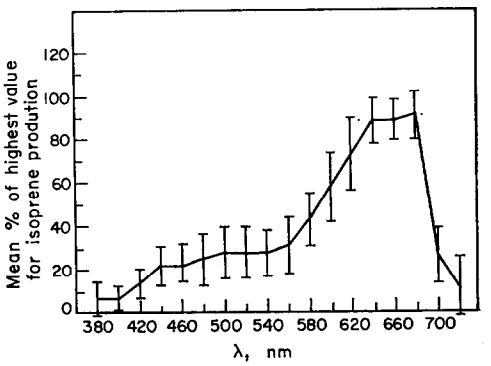

FIG. 4. ACTION SPECTRUM FOR ISOPRENE EMISSION.

Discs were cut from leaves in the dark, floated on water at 19 ± 2° in the different wavelengths of light for 15 min, then enclosed for 2 hr and isoprene measured. $N = 8$ leaf discs. Bars represent 1 s.d. Headspace corrected to 6 ml.

An action spectrum for the production of isoprene from *Hamamelis* leaf discs is shown in Fig. 4.

Lack of a 'blue peak' in the action spectrum of isoprene production was observed. Other studies have also found photosynthetic action spectra in leaf tissue with only a 'red peak'.[9]

TABLE 1. RATES OF ISOPRENE EMISSION IN LEAF DISCS

Temp. (°)	*Quercus* $\bar{x}$	*Quercus* σ	*Eucalyptus* $\bar{x}$	*Eucalyptus* σ	*Hamamelis* Old $\bar{x}$	*Hamamelis* Old σ	*Hamamelis* New $\bar{x}$	*Hamamelis* New σ
15	0·15	0·04	0·10	0·06	0·09	0·15	0·09	0·03
25	0·65	0·08	0·58	0·15	0·54	0·11	0·95	0·24
35	2·35	0·20	1·82	0·29	1·67	0·47	3·2	0·51
40	2·49	0·52	1·94	0·43	2·21	0·77	4·4	0·53
45	1·58	0·27	0·14	0·17	1·72	0·70	3·9	0·58
Q_{10}	3·6		3·2		3·1		3·4	

Isoprene production in ppm emitted into 1 ml headspace in 1 min by 1 cm^2 tissue. Q_{10} for (25–35°). $N = 9$. Oak, *Quercus borealis*; Eucalyptus, *E. Gunii montana*; Witch Hazel, *Hamamelis jelena*.

It can be hypothesized that the lack of a characteristic blue peak in photosynthetic action spectra is due to excess photosynthetically inactive pigments which absorb in the blue. This has been correlated with absorption spectra in abnormally dense leaves which transmit almost no incident light at any wavelength.[9] To test this correlation, the absorbance of a

[9] S. LINDER, *Physiol. Plant.* **25**, 58 (1971).

typical shade-grown *Hamamelis* leaf was determined (Fig. 5). The absorbance is very high with less than 1% of incident light transmitted at any wavelength. Therefore, the action spectrum for isoprene production is consistent with reported photosynthetic action spectra for other thick, dense leaves.[9]

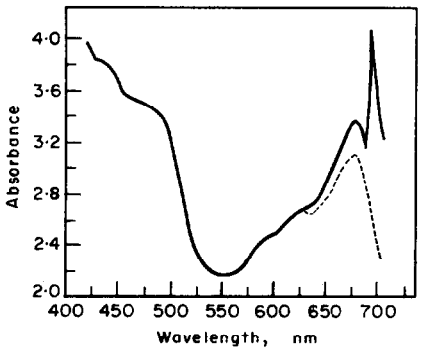

FIG. 5. ABSORBANCE OF A TYPICAL *Hamamelis* LEAF.
Dotted line indicates infra-red lamp source normally used above 625 nm. Browning of the leaf occurred with this source; therefore, the trace produced by the visible lamp is shown as a solid line.

The effect of dichlorodimethylphenylurea (DCMU, 10^{-4} M), thought to block electron flow in photosystem II, and 2,4-dinitrophenol (DNP, 10^{-3} and 10^{-4} M), a powerful uncoupler of ATP production via oxidative phosphorylation, on the production of isoprene

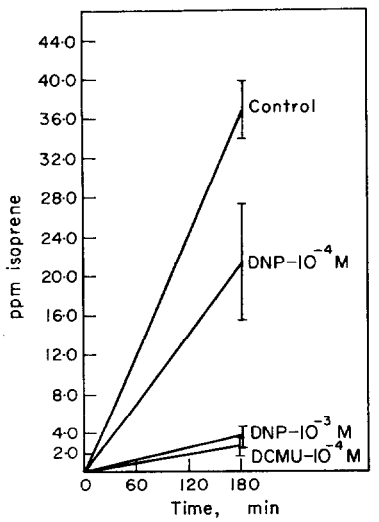

FIG. 6. EFFECT OF DCMU AND DNP ON PRODUCTION OF ISOPRENE.
$N = 7$, headspace corrected to 10 ml.

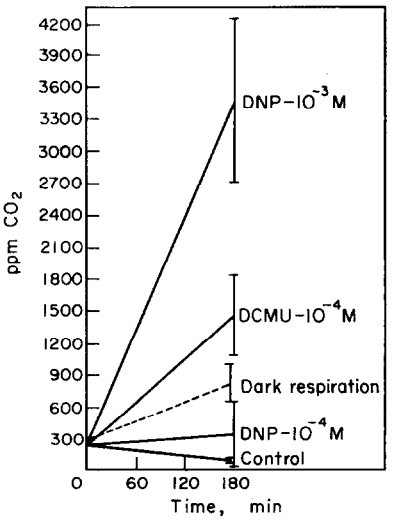

FIG. 7. EFFECT OF DCMU AND DNP ON PRODUCTION OF CO_2.
$N = 7$, headspace corrected to 10 ml.

is shown in Fig. 6. Leaf discs were stirred in the respective solutions for 1 hr in order to effect infiltration of the tissues, then left for 3 hr in the solutions while the rate of emission

of isoprene was studied. The results show that both DCMU at 10^{-4} M and DNP at 10^{-3} M strongly inhibit isoprene production.

Simultaneous measurements of CO_2 concentration (Fig. 7) showed DNP (10^{-3} M) and DCMU (10–^{4} M) raised CO_2 production well above the normal dark respiration rate. DNP produces this effect primarily by uncoupling ATP. DCMU has been reported to permit mitochondrial processes to operate in the light[10] by reversing the photo-inhibition of dark respiration. In addition, it inhibits photosynthesis and can stimulate photorespiration. DCMU could therefore be affecting one or more of these processes in *Hamamelis* leaf discs.

The effects of different CO_2 concentrations on the emission of isoprene is likewise unclear. Tentative results indicate that high CO_2 concentrations reduce isoprene emission both through stomatal closure and genuine inhibition of isoprene production. CO_2 concentrations reduce isoprene emission both through stomatal closure and genuine inhibition of isoprene production. CO_2 concentrations below the compensation point initially stimulate isoprene emission but eventually inhibit production due to depletion of photosynthate. These effects will be reported in a subsequent paper.

EXPERIMENTAL

The concentration of isoprene was measured using a Carle Model 9000, dual column GLC, with flame ionization detectors equipped with 1·8 m, 0·32 cm o.d., 4% Carbowax 20 M on Chromosorb W, HP, 60–80 mesh columns held isothermally at 76–81°. Injection of the gas samples was made with a Pressure-Lok® syringe of 0·5 ml capacity using pressurized injection technique. Experimental error in sampling and analysis was less than ±5%. Isoprene concentrations as low as 0·5 ppm in 0·2 ml air samples could be measured. The concentration of carbon dioxide was measured using a Carle Model 8004, GLC with dual thermal conductivity detectors using 1·8 m, 0·32 cm o.d. Porapak Q 60–80 mesh columns at 56°. CO_2 concentrations to 50 ppm in 0·2 ml air samples could be measured accurately with this system. Isoprene and CO_2 analyzed reference gases were obtained from Scott Research Laboratories for calibration purposes.

Hamamelis jelena (Hamamelidaceae) plants were obtained from Staneck's nursery, Spokane, Washington. Some plants were bought early in the spring and grown under strong light (11 000 lx) in a growth chamber, 12 hr light, 28°. Others were grown until late summer in diffuse sunlight, then placed in the growth chamber. Leaf discs (3·8 cm^2) were cut from fresh, light-acclimated *Hamamelis* leaves on either side of the midvein. Temperature was regulated within ±1° by floating the leaf discs on water in 10 cm diameter petri dishes placed in constant temperature baths. The anisolateral leaf discs were placed abaxial side up on distilled water and allowed to acclimate to those conditions for at least 15 min prior to being studied. The discs were then enclosed in cylindrical Lucite® chambers of 15 ml capacity by placing the open end of the chamber over the disc and into the water. Immediately after the leaves were enclosed, air was withdrawn from the chamber with a syringe through a septum, until a standard air volume of 'headspace' was obtained. Gas samples, normally from 0·2 to 0·4 ml were removed similarly. In cases when more than one sample was removed from a chamber, a correction in calculating the subsequent concentrations of isoprene was made for the reduced volume of the chamber.

Determination of action spectrum. Details concerning the operating conditions of the xenon arc diffraction grating spectrograph as well as the method of obtaining the spectral energy distribution are given by Balegh and Biddulph (1970).[11] Isoprene emission was assumed to be linearly related to the intensity of incident light at the quantum irradiances used, and the measured isoprene concentrations were adjusted to a standard light intensity of $5{\cdot}0 \times 10^{16}$ photons cm^{-2} sec^{-1}. Sixteen discs from a single leaf were used in each trial to examine the effects of 16 different wavelengths. Considerable inter-leaf variation was found. Consequently the data was processed as follows. Each 2-hr trial was examined and the concentration of isoprene produced by the most rapidly emitting leaf disc in that trial was set equal to 100%. All other values obtained within that trial were ranked as percentages of the highest value. The percentages obtained for each wavelength were averaged and are shown as the 'mean % of the highest value for isoprene production'.

Acknowledgements—We would like to thank Dr. Joseph E. Scheibe for his interest and criticism and Dr. Merle G. Kleinschmidt for furnishing DCMU and DNP. This project has been financed in part with Federal funds from the Environmental Protection Agency under grant number AP 01115.

[10] W. A. Jackson and R. J. Volk, *Ann. Rev. Pl. Physiol.* **21**, 385 (1970).
[11] S. E. Balegh and O. Biddulph, *Plant Physiol.* **46**, 1 (1970).