Comparison and Evaluation of Methods for the Removal of Ethylene and Other Hydrocarbons from Air for Biological Studies[1]

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ABSTRACT

A random sampling analysis of laboratory air and of air from commercially available cylinders indicated that they contain appreciable amounts of low molecular weight hydrocarbons, viz. methane, ethane, and ethylene, as contaminants. These impurities could lead to erroneous conclusions in studies of plant growth and metabolism. Different methods for removal of these contaminants were compared and evaluated in the present investigation for their suitability in plant studies. Most of the methods currently being used were found inadequate. The use of metal catalysts at high temperature, adapted from gas analysis techniques, provides an inexpensive and efficient method for removing hydrocarbons from air in both closed and continuous flow systems.

The effects of various constituents and contaminants of air on plant metabolim have been studied for many years (10). The possible roles of light hydrocarbons have been of particular interest in this regard, and ethylene is now recognized as a plant hormone. Ethylene has been implicated in most phases of plant growth, for example seed germination, hook opening, hypocotyl elongation, sex expression, fruit ripening, senescence, and abscission (1), and very low concentrations of ethylene are sufficient to initiate a number of these responses.

Most work on the effects of ethylene has been conducted with closed systems (5, 11, 26). A closed system is usually selected for convenient handling and gas sampling. However, since plant tissues produce ethylene, in closed systems they will be exposed to constantly changing ethylene concentrations in addition to changes in O_2 and CO_2 levels. This inadequate control of gas composition could lead to erroneous conclusions. Even in studies not aimed at the study of effects of ethylene, this variation in ethylene concentration could be of crucial importance. The interactions between ethylene and CO_2, for instance, have been well documented (12, 13). Thus, a continuous flow system to prevent accumulation of abnormal gas concentrations is desirable.

Even in a continuous flow system, hydrocarbons present as impurities in the air supply may affect plant metabolism. In our analysis, random samples of laboratory air and several cylinders of commercially available air mixtures were found to contain appreciable and differing concentrations of hydrocarbons. It has already been demonstrated that ambient concentrations of ethylene are able to elicit certain responses in plants (2). It remains to be seen whether ambient levels of other hydrocarbons such as

methane or ethane can also affect plant metabolism either alone or through their interaction with ethylene. Little effort has been made to purify air with respect to these hydrocarbons in controlled systems being used in studies of plant physiology. Although some methods have been proposed to reduce levels of ethylene selectively, the problem of contamination of air streams by other hydocarbons persists.

The present investigation addresses these problems. We have evaluated different methods available for purification of air for biological studies.

MATERIALS AND METHODS

A commercially available cylinder of compressed air was used for all experimental procedures reported in this paper. Our analysis indicated that it contained 9.16 μl/l methane, 0.020 μl/l ethylene, and 0.420 μl/l ethane. Other cylinders gave similar results but because comparisons among methods are to be made, figures are included only for samples from one cylinder. The pressure was adjusted through a two-stage regulator to establish an air flow of 100 ml/min through each purification system. All connections and tubing were made of metal or glass. Samples were taken from the effluent air through a minimum bleed septum fitted in the air line.

A survey of the literature suggested several approaches to air purification. Table I summarizes the methods compared in this study. The adsorbents (methods 1–9) were packed in a copper U-tube (45 × 1.27 cm o.d.). The packing materials were activated at 200 C for at least 16 hr before being used. A temperature of −86 C was attained by a dry ice-acetone slurry and −196 C by liquid N_2. The liquid traps (methods 10 and 11) consisted of 10 ml of reagent in a small gas-washing tube; the air was dispersed through a fritted glass aerator. The solid reactive materials (methods 12–22) were packed in a stainless steel tube (45 × 1.27 cm o.d.). Elevated temperatures, when required, were attained with a semimicrocombustion furnace (Sargent-Welch Scientific Co.) with a heating chamber 18 cm long. Coconut charcoal (method 12) was brominated by slowly adding bromine to the charcoal in a stoppered flask. Brominated charcoal was followed by activated coconut charcoal in the second half of the column to absorb any residual bromine. When potassium dichromate (method 13) and potassium permanganate (methods 14 and 15) were used as active reagents, they were added to the silica gel as a 40% (w/v) suspension in water, then dried at 200 C overnight. Chromotropic acid (4,5-dihydroxy-2,7-naphthalenedisulfonic acid) was deposited on silica gel (method 15) in the same way and then dried at 60 C *in vacuo* for 3 hr. Finely powdered iodine pentoxide (method 16) was added directly to the silica gel. Selenium oxide reagent (method 17) was prepared by adding selenious acid to silica gel and heating to 200 C overnight. Sublimation to yellow crystals in the stoppered flask indicated the formation of selenium oxide. Silver nitrate was

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Table I. Summary of materials and methods compared for their ability to remove hydrocarbons from an air stream. The preparation of the reagents and the assembly of the apparatus is described in the text.

Method Number	Description	Temp. (C)	Ref.
1	Cocoanut charcoal (50 - 200 mesh)	22	23
2	Cocoanut charcoal (50 - 200 mesh)	-86	23
3	Cocoanut charcoal (50 - 200 mesh)	-196	23
4	Silica gel (30 - 60 mesh)	-86	22
5	Natrasorb T (4 - 12 mesh)	-86	--
6	Molecular Sieve 13X (60 - 80 mesh)	-86	23
7	Molecular Sieve 13X (60 - 80 mesh)	-196	23
8	Davison Molecular Sieve, 0.3 nm pore size (4 - 8 mesh)	-86	23
9	Davison Molecular Sieve, 0.3 nm pore size (4 - 8 mesh)	-196	23
10	40% aqueous formaldehyde in concentrated sulphuric acid (3.3 : 100)	22	25
11	40% aqueous formaldehyde in fuming sulphuric acid (3.3 : 100)	22	--
12	Brominated cocoanut charcoal (20% w/w) followed by cocoanut charcoal	22	20
13	20% (w/w) potassium dichromate on silica gel with 5% (v/w) fuming sulphuric acid	22	14
14	20% (w/w) potassium permanganate on silica gel with 5% (v/w) fuming sulphuric acid	22	6, 7
15	20% (w/w) potassium permanganate on silica gel with 5% (v/w) fuming sulphuric acid followed by 20% (w/w) chromotropic acid on silica gel	22	6
16	20% (w/w) iodine pentoxide on silica gel with 5% (v/w) fuming sulphuric acid	200	3, 24
17	20% (w/w) selenium oxide on silica gel with 5% (v/w) fuming sulphuric acid	22	9
18	5% (v/w) fuming sulphuric acid on silica gel	22	24
19	2% (w/w) silver nitrate on silica gel	22	17
20	30% (w/w) cupric oxide, 0.3% (w/w) ferric oxide in Kieselguhr pellets	800	16, 19, 24
21	50% (w/w) cupric oxide powder on Chromosorb P (42 - 60 mesh)	800	8, 18
22	5% (w/w) platinum on asbestos fibres	650	15, 16

Table II. The relative concentrations of three hydrocarbons in an air stream subjected to different purification procedures. Time 0 refers to the time when the first samples were taken after 10 minutes of flushing. The method numbers correspond to the numbers given in Table I.

Method Number	Hydrocarbon Concentration (per cent of control[a])								
	Time of Sampling (minutes)								
	0			30			60		
	Methane	Ethane	Ethylene	Methane	Ethane	Ethylene	Methane	Ethane	Ethylene
Control	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
1	92.6	0	0	91.2	0	0	100.8	0	0
2	8.8	0	0	7.3	0	0	15.1	0	0
3	0.6	0	0	0.6	0	0	0.5	0	0
4	3.2	0	0	100.6	0	0	99.1	0	0
5	59.8	0	0	100.0	0	0	100.5	0	0
6	40.1	0	0	98.6	0	0	101.3	0	0
7	2.1	0	0	2.7	0	0	30.1	0	0
8	97.8	13.3	0	100.0	97.1	0	100.4	104.3	0
9	74.4	0	0	55.6	0	0	89.1	0	0
10	96.4	94.8	75.0	95.7	99.8	80.0	98.1	94.8	80.0
11	96.1	89.8	0	97.8	102.4	b	97.2	97.6	75.0
12	93.9	0	0	95.3	0	0	97.5	b	0
13	96.2	92.1	0	94.2	95.7	0	93.6	96.7	0
14	97.4	96.7	0	99.1	101.7	0	99.0	102.1	0
15	98.9	71.7	0	99.9	79.0	0	100.4	73.6	0
16	96.5	58.6	0	93.8	b	0	89.5	0	0
17	97.3	98.6	0	96.8	102.6	0	95.4	96.2	0
18	100.1	99.0	60.0	101.0	105.2	65.0	99.3	103.1	70.0
19	100.1	102.1	0	104.0	100.0	0	97.9	103.8	0
20	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0	0

a The concentration of methane, ethane and ethylene in the control air supply is 9.16 µl/1, 0.420 µl/1 and 0.020 µl/1 respectively. These concentrations are regarded as 100.0% for ease of comparison.

b The hydrocarbon peak on the gas chromatogram is detectable, but below the limits of integration.

deposited on silica gel as 4% (w/v) solution and dried at 90 C for 3 hr (method 19). Fuming (30% SO₃) or concentrated H₂SO₄, when used in any of these methods, was added immediately before packing the column.

Cupric oxide (copper (II) oxide) pellets (method 20) were prepared essentially as described by Schenk (19) with the following modifications. Ferric oxide (iron (III) oxide) powder was added to the cupric oxide/Kieselguhr paste to improve the efficiency of the catalyst (24). The mixture was extruded through a syringe and dried at 200 C to form pellets (0.2 × 0.8 cm). The reduction of cupric oxide with hydrogen (19) was omitted.

In each case, the apparatus was allowed to reach temperature equilibrium before the flow rate was established. The system was then flushed for 10 min before the first samples were withdrawn. This time, immediately after the completion of the 10 min of flushing, is designated as time 0 in Table II. Samples were taken at two additional times at 30-min intervals to assess the stability of each method. At each designated time, three 5.0-ml samples were withdrawn in 5 ml syringes. Each syringe was flushed with sample air five times before taking the sample and the time between sampling and analysis was kept to a minimum. We found that the mere penetration of the septum by the needle could result in an appreciable peak that coincided with the methane peak. A finer needle (26 gauge; 1.27 cm long) overcame this problem.

Hydrocarbons and CO₂ were analyzed in a Model 5830A Hewlett Packard gas chromatograph equipped with side-port valves. Various constituents of air were separated through a stainless steel column (300 × 0.3175 cm o.d.) packed with Porapak Q (80–100 mesh, Waters Associated Inc.) run isothermally at 40 C. Hydrocarbons were detected by a flame ionization detector and CO₂ by a thermal conductivity detector. The limit of detection (signal to noise ratio ≈1) was 0.05 ng of each hydrocarbon. The gas chromatograph was equipped with an electronic integrator (sensitivity limit 0.5 ng) that was used to quantify the peaks. For smaller amounts, the peak areas were determined by height-width measurements. All areas were converted to µl/1 by comparison with analyzed air mixtures from Linde Specialty Gases. These mixtures were checked against independently analyzed standards.

When a more sensitive means of detecting ethylene was required, the effective sample size was increased 300-fold by use of a collection system (22). A four-way gas-sampling valve was attached to a stainless steel U-tube (16 × 0.635 cm o.d.) packed with 1.5 g of silica gel. The use of a valve was necessary to exclude room air from the U-tube. This amount of room air was found to

contribute detectable amounts of hydrocarbons. The trap was flushed with the sample gas for 10 min while immersed in boiling water to prevent retention of ethylene. The valve was then set to bypass the trap. The trap was immersed in a dry ice-acetone slurry and allowed to equilibrate for 2 to 3 min. The air flow was then directed through the trap for 15 min to collect any ethylene present in the air stream. The collection apparatus, still immersed in dry ice-acetone, was attached to the side-port of the gas chromatograph and the flow of carrier gas was established through the side-port connections. The trap was heated to 60 C for 10 min to release ethylene from the silica gel. Then the carrier gas was directed through the U-tube to carry the liberated ethylene to the column of the gas chromatograph.

The O₂ concentration of the gases was determined by the use of a portable O₂ analyzer (model D2, Arnold A. Beckman Inc., South Pasadena, Calif.).

Suppliers. Natrasorb T is an adsorbent distributed by Cullen Industries Inc., Buffalo, N. Y. Five per cent platinum on asbestos was obtained from K & K Laboratories Inc., Plainsview, N. Y. The coconut charcoal was from Fisher Scientific Co. The silica gel used throughout this study was 30 to 60 mesh and obtained from Matheson, Coleman and Bell Manufacturing Chemicals.

RESULTS AND DISCUSSION

The results are assembled in Table II. It is evident from the data that of the three hydrocarbons considered, methane was most difficult to remove from the air stream. Because methane is less reactive and more volatile (boiling point, −165 C) than ethane or ethylene, most oxidative and adsorptive approaches are ineffective.

The primary limitation of adsorbent traps is the saturation of their binding capacity, leading to subsequent loss of efficiency. This point is illustrated by the silica gel trap maintained at −86 C (method 4). Although immediately after the initial flushing period of 10 min (time 0, Table II) the trap was retaining 96.8% of the methane, after an additional 30 min it had become completely ineffective in this respect. Nonetheless, both ethylene and ethane were being completely removed throughout the experiment. Of the adsorbent traps considered, activated coconut charcoal at

−196 C was the most effective within the time frame of this experiment. This trap would also be subjected to the drawback of finite binding capacity with extended use.

The silica gel trap (method 4) presented an added problem by retaining CO_2 from the room air initially in the trap. After 70 min of flushing with sample gas, the CO_2 concentration of the effluent air had declined from an initial value of 392.2 $\mu l/l$ to 9.5 $\mu l/l$ and still had not equilibrated. Although retention of CO_2 was observed in several cases, the problem was generally more pronounced in purification methods utilizing a silica gel solid support. CO_2 concentration should therefore be carefully monitored while using any of these systems.

O_2 (boiling point, −183 C) tends to condense out of the air stream in adsorbent traps at −196 C (21). After 10 min of flushing (time 0), the O_2 concentration of the effluent from the activated charcoal trap at −196 C had declined to 1.4% from the control value of 19.9%. The O_2 concentration of subsequent samples from the same trap increased rapidly as the trap approached equilibrium. As the liquid O_2 accumulated, however, the air flow became very erratic.

All the chemical reactants (methods 10–22) effectively removed ethylene from the air stream with the exception of the methods where H_2SO_4 was mixed with aqueous formaldehyde (methods 10 and 11) or deposited on silica gel (method 18). When fuming H_2SO_4 was substituted for the concentrated H_2SO_4 in aqueous formaldehyde as originally suggested (25), no ethylene was detectable in the samples taken at time 0. Subsequent samples contained an appreciable amount of ethylene. The incomplete removal of ethylene by brominated activated charcoal (7, 20) and permanganates (7) reported in previous studies, as compared to our results (methods 12, 14, and 15), could be a consequence of different surface to volume ratios and different initial amounts of ethylene. Iodine pentoxide (method 16) oxidized ethylene as well as most of ethane. The consequent reduction of iodine pentoxide to molecular iodine, which is bright red, could be used as an indicator to assess exhaustion of the trap. A consistent limitation of the methods 10 through 19 is their inability to completely remove methane.

Table II clearly shows that platinized asbestos, cupric oxide-ferric oxide pellets, and powdered cupric oxide maintained at high temperatures (methods 20–22) provide the most efficient means of removing hydrocarbons from a continuous air flow system without creating the problems encountered with adsorbent traps. These catalysts depend upon a constant supply of O_2 for regeneration. In the air supply used for this comparison, O_2 was present at a sufficiently high concentration to maintain the active catalysts. A stoichiometric amount of O_2 will, of course, be removed from the air stream. In the present study, the O_2 concentration was reduced from 19.9% in the control to 19.1% in the air that had been passed through the platinized asbestos column (method 22). Similar small reductions in O_2 concentrations were noticed with cupric oxide catalysts (methods 20 and 21). The longevity of the catalyst systems was indicated by the fact that no loss of efficiency could be detected after 24 hr of continuous operation at 100 ml/min. In our laboratory, similar purification systems have been in use for several months without any appreciable deterioration. The amount of CO_2 produced by oxidation of hydrocarbons should also be considered. The effluent from the platinized asbestos catalyst contained 12.3 μl of CO_2/l of purified air whereas the CO_2 levels of the control air were below the limits of integration (Fig. 1). The amount of CO_2 produced will vary depending on the concentrations of hydrocarbon impurities in the air being used.

We have also assessed the relationship between flow rate and catalytic efficiency of platinized asbestos (5%) heated to 650 C. Figure 2 shows the results of this experiment. As the flow rate was increased beyond 200 ml/min, the concentration of methane in the air started to go up. No ethane or ethylene was detected even at 800 ml/min. The ability of the catalyst to oxidize methane
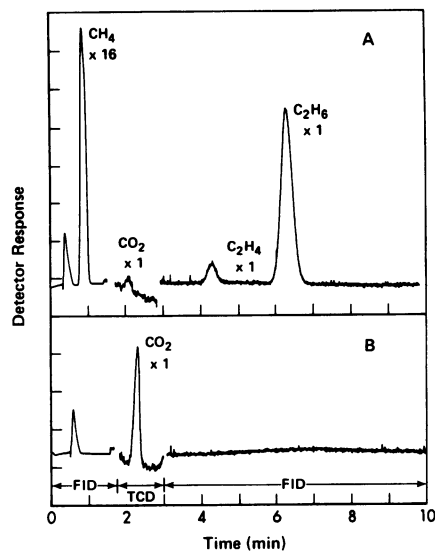

FIG. 1. Gas chromatograms illustrating the effect of heated catalysts on CO_2 and hydrocarbon concentrations in an air stream. Concentrations of CH_4, C_2H_4, and C_2H_6 were 9.16 $\mu l/l$, 0.020 $\mu l/l$, and 0.420 $\mu l/l$, respectively, in control air (A). There were no detectable hydrocarbons in the air that had passed through the platinized asbestos catalyst heated to 650 C (B), but the concentration of CO_2 increased to 12.3 $\mu l/l$.
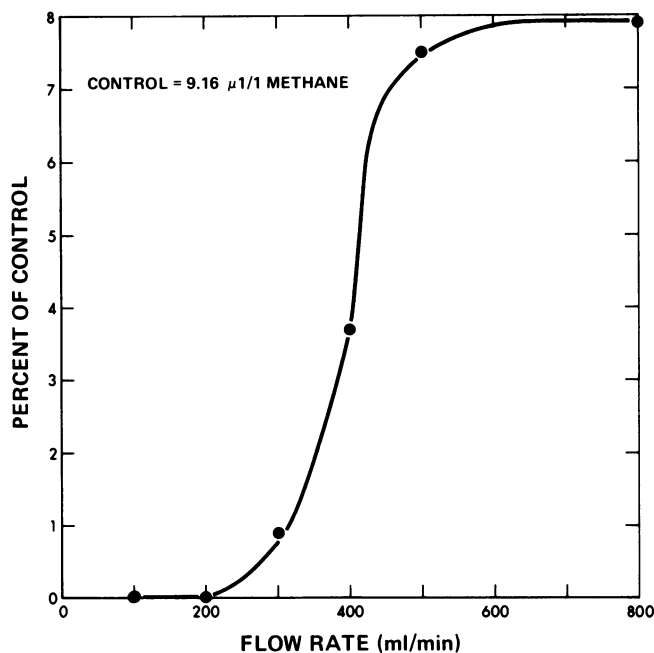

FIG. 2. Relationship between flow rate and ability of a heated platinum catalyst (method 22) to oxidize hydrocarbons from contaminated air supply. No ethylene and ethane were detectable even at 800 ml/min.

completely at this high flow rate could be regained by increasing the operating temperature to 800 C. However, such high temperatures led to considerable deplatinization of asbestos in the column after prolonged use. It is important to recognize that the efficiency of all catalysts is a function of the ratio of surface area to air volume, and the maintenance of the appropriate uniform temperature.

Some of the methods suggested in the literature for removing ethylene from air have not been included in this study. Mercuric perchlorate is commonly used to scrub ethylene from air (27); this method involves problems related to the reagents' corrosive nature and to the disposal of heavy metal wastes. The efficiency of the

method is more dependent on flow rates and degree of foaming of the mercuric perchlorate (4) than is generally realized. The slow reaction rates and the amount of equipment involved in methods using ozone or x-rays preclude their general use for plant studies.

The materials used in systems for biological studies should be chosen carefully. Plastics and rubbers are known to contaminate the air stream by evolving low mol wt hydrocarbons. This problem is accentuated by light and high temperature. We have also found that these materials can absorb hydrocarbons, depending upon the composition of the air mixture.

CONCLUSIONS

The results of this investigation illustrate the need for more rigorous control of the air used in plant physiology studies. Common sources of air contain appreciable concentrations of several hydrocarbons, including ethylene, a known plant growth regulator.

The high temperature catalysts can oxidize the light hydrocarbons present in most air supplies without many of the problems associated with other techniques. The catalysts tested (methods 20-22) can be used in various forms. Platinum is commercially available in a variety of concentrations on several different solid support materials, which may be in the form of granules, pellets, fibers, or powders. Similarly, cupric oxide can be used in each of these forms. Pellets offer relatively less resistance to air flow, but only at the expense of surface area. The catalyst systems can be assembled inexpensively. We used a semimicro combustion furnace in the present comparisons for stability and versatility. However, high temperature heating tapes are currently being used in the laboratory very successfully.

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