MEASUREMENT OF SMALL CONCENTRATIONS OF ETHYLENE AND AUTOMOBILE EXHAUST GASES AND THEIR RELATION TO LEMON STORAGE

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(WITH FIVE FIGURES)

Introduction

Ethylene, even in very small concentrations, is known to hasten the coloring and ripening of fruits and vegetables in storage and to greatly increase their rate of respiration.

Further study of this problem has been made for the purpose of obtaining a satisfactory test for the detection of small concentrations of ethylene in connection with commercial storage of lemons. It was desirable to know what concentrations of ethylene accumulate in lemon storage rooms from decaying fruit; also what might be the importance of motor exhaust gases from loading and unloading trucks or from the cars and trucks of nearby highways.

Probably the earliest written record of the effects of motor gases on fruit is given in a paper by SIEVERS and TRUE (**17**). They reported that lemons were being colored quickly in the packing house by piping the exhaust gases from a gasoline engine into the coloring rooms. They also stated that the exhaust gases produced a very satisfactory fruit color in the same time as was required with kerosene stove fumes. Apparently the fruit was colored as quickly as it now is with ethylene.

WRIGHT (**18**) describes methods of using motor exhaust gases for coloring oranges. This author states that the gas in a well-regulated coloring room should be just strong enough that a person experiences only a slightly disagreeable odor or burning sensation of the eyes. His observation was that the first sensation experienced on entering the room is the most reliable. He recommends that the gas be run into the room during only about 10 hours a day.

BARGER and HAWKINS (**1**) have described the satisfactory use of gasoline engine exhaust fumes for coloring grapefruit in Florida. DENNY (**5**) after analysis of kerosene stove fumes concluded that the ethylene fraction was mainly responsible for the coloring of fruit and stated that ethylene when used in concentrations as low as 1 p.p.m. brings about this coloring. He also pointed out that a wide range of ethylene concentrations from 1 part in 2 million to 1 part in 5000 may be used to color fruit.

MACK (**14**), working on the blanching of celery with ethylene, found that 20 p.p.m. to 40 p.p.m. gave the optimum concentration for blanching and concentrations more than 40 p.p.m. gave less blanching.

It has been shown that a number of different fruits give off ethylene gas during ripening. While ELMER (**6**) appears to be the first to point out that apples give off some form of physiologically effective gas, it remained for GANE (**7**) to prove that the gas was ethylene. Other fruits have also been found to give off ethylene. NIEDERL *et al.* (**16**) found that bananas give off small amounts of ethylene while ripening. HANSEN and HARTMAN (**10**) in 1935 gave considerable evidence that ethylene was given off by pears. HANSEN (**9**), again working on pears and measuring the ethylene by chemical means, studied the relation of ethylene production to respiration. He found that the amount of ethylene given off by the ripening of pears was influenced greatly by such factors as temperature, anaerobic conditions, and varietal differences. BIALE (**2**) and MILLER *et al.* (**15**) have shown that emanations, believed to be ethylene, are given off by citrus fruit decaying from the common green mold, *Penicillium digitatum* Sacc. MILLER *et al.* (**15**) also found that sound citrus fruit gave slight but positive tests for ethylene.

Without having some delicate simple test, it is impossible to know when storage rooms are properly ventilated or whether the air being taken into the storage rooms is free from ethylene. It has been our purpose, therefore, to develop a method which could be used to test automobile exhaust gases for ethylene and to detect small amounts of this gas in fruit storage rooms.

Method

The epinasty test using pea plants, with some modification of the method as described by KNIGHT *et al.* (**12**) and KNIGHT and CROCKER (**11**), seemed to offer the best possibility as a method of quantitatively measuring very small ethylene concentrations.

Results

MEASUREMENT OF THE ETHYLENE EQUIVALENT OF MOTOR EXHAUST FUMES

Alaska peas were planted in cans $2\frac{1}{2}$ inches high and 4 inches in diameter, the cans being filled with one part sand, two parts water and three parts peat moss. The peas were grown at 75° F. until the plants were one or two inches high. This usually required four days.

One can of peas was placed in each of eight removable lid steel drums of 55-gallon capacity. After the peas were placed, the lids were clamped tightly and different measured quantities of motor exhaust fumes were placed in five of the drums; measured quantities of ethylene were placed in two drums, and one was kept as a control. After four days, peas grown in the exhaust fumes were compared with the others grown in the drums with known quantities of ethylene and with the control to which no gas had been added.

The automobile exhaust gases to be tested were first introduced into an ordinary steel drum which served only as a reservoir. This was done by means of a $1\frac{3}{4}$-in. hose one end of which was placed on the exhaust of a Ford V8 automobile and the other end into the side opening of the drum. The top of this drum contained a four-inch opening from which the gases could escape. The automobile motor was started and the gas fumes allowed to run into the drum for about 15 minutes to displace the air. The rate of flow of the gas from the motor was measured by having an anemometer on the opening in the top of the drum and the quantity of exhaust gases coming from the motor in a given time was recorded. A single barrel tire pump had been made into a syringe by removing the valves, placing a double leather on the plunger and calibrating it. The pump was used to remove given quantities of the motor gas from this drum and place it in the drums containing peas.

A known amount of ethylene was introduced into two of the drums as follows. The ethylene was first introduced into a 5-gallon carboy to give a

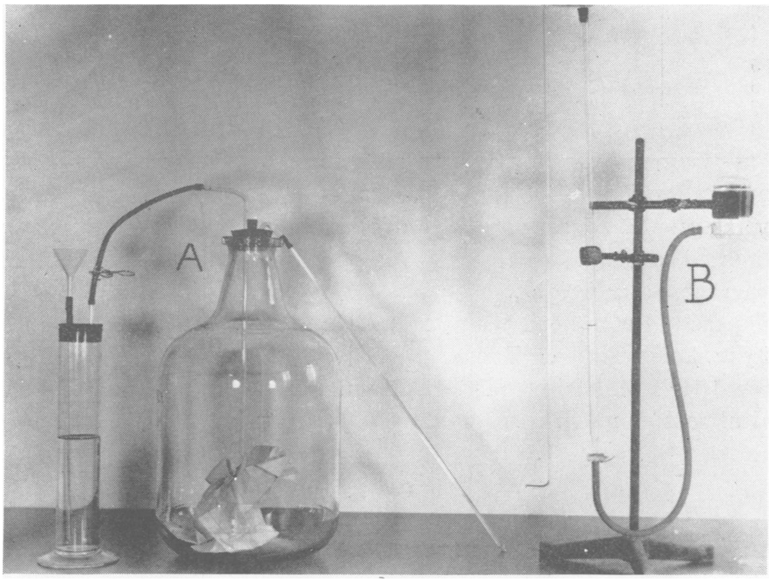

FIG. 1. Equipment used for measuring out small quantities of ethylene into epinasty test drums.

concentration of 1 part ethylene to 2000 parts air mixture. This was done by means of water displacement from a burette having a 0.5-mm. capillary tube connected at the top and a leveling bottle at the bottom. The leveling bottle was raised to fill the burette and capillary with water. Then the capillary was attached to the ethylene container and the water in the capillary and most of that in the burette were displaced with ethylene. The capillary was then connected to the carboy and the desired amount of ethylene introduced by raising the leveling bottle (see fig. 1B). The carboy also contained a piece of crumpled paper which helped to mix the gas contents when the carboy was shaken. The desired amount of this diluted gas was then introduced into the barrels containing the pea plants by connecting a closed graduated cylinder with a funnel and a capillary tube. The capillary tube was inserted into the bung hole of the drum. By adding a given quantity of water to the graduated cylinder air was forced into the carboy, displacing an equal amount of the gas mixture from the carboy into the drum with the peas. Direct water displacement was avoided since ethylene is appreciably water-soluble (see fig. 1A).

The ethylene and the concentrations obtained in the drums are given in table I.

TABLE I

Drum number	Gas added	Amount of gas added	Concentration in drum
		ml.	*p.p.m.*
1	None	....	....
2	Ethylene	0.05	0.25
3	Ethylene	0.1	0.5
4	Motor exhaust	25.0	120.0
5	Motor exhaust	80.0	400.0
6	Motor exhaust	160.0	800.0
7	Motor exhaust	400.0	2000.0
8	Motor exhaust	800.0	4000.0

Figure 2 shows a set of peas after four days in these drums. There was no bending due to epinasty in the control drum although the plants were tall and etiolated so that when removed for photographing several of them bent over from their own weight.

The peas in drum no. 2 with 0.25 p.p.m. of ethylene bent over and grew 2″ to 2½ in. horizontally. Those in no. 3 with 0.5 p.p.m. ethylene bent over and grew 1½ to 2 in. horizontally. Those in no. 4 with 120 p.p.m. of motor gas showed almost as much epinasty as those in 0.25 p.p.m. of ethylene. The

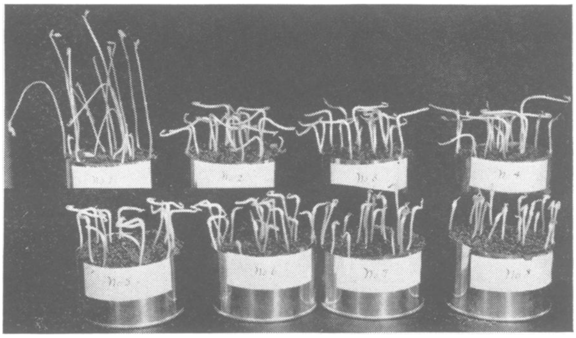

FIG. 2. Epinasty produced by ethylene and motor exhaust gases on Alaska peas.

No. 1. Control (several peas bent over by their own weight after removing from test drum).
No. 2. 0.25 p.p.m. ethylene
No. 3. 0.5 p.p.m. ethylene
No. 4. 118 p.p.m. motor exhaust
No. 5. 400 p.p.m. motor exhaust
No. 6. 800 p.p.m. motor exhaust
No. 7. 2000 p.p.m. motor exhaust
No. 8. 4000 p.p.m. motor exhaust

peas in drums nos. 5, 6, 7, and 8 showed progressively less plant growth. No. 8 showed almost no growth at all. The last four showed considerable swelling or enlargement of the stems.

From these results it appears that no. 4, with 120 p.p.m. of motor exhaust gas, was affected slightly less than no. 2 with 0.25 p.p.m. of ethylene. No. 5, with 400 p.p.m. of exhaust gas, was affected much more than no. 3 with 0.5 p.p.m. of ethylene. It would appear that drum no. 5 has about twice as high a concentration of ethylene as no. 3.

If we consider that no. 4 with 25 ml. of motor exhaust gas is the equivalent of no. 2 with 0.05 ml. of ethylene and that no. 5 has twice the ethylene equivalent of no. 3 we can calculate the ethylene equivalent of the motor fumes as follows:

0.05 ml. ethylene = 25 ml. motor exhaust gas.
1 ml. ethylene = 500 ml. motor exhaust gas.
2000 ml. ethylene = 1,000,000 ml. motor exhaust gas or 2000 p.p.m.

The peas in no. 4 did not bend as sharply, and they grew a little longer than those in no. 2, indicating that the ethylene equivalent in no. 4 was not quite as great as that of no. 2. The ethylene equivalent of the motor gas in barrel no. 4 was probably more nearly equal to 0.04 ml. of ethylene; and assuming that the ethylene quivalent of the exhaust gas in the air in barrel no. 4 was equal to 0.04 ml. ethylene, then the exhaust gas has an ethylene equivalent of 1600 p.p.m. instead of 2000 p.p.m. The ethylene equivalent of the motor gas is then in the range of 1600 to 2000 p.p.m.

It was found that the car gave off 529 liters of exhaust gas per minute when idling. If this gas has an ethylene equivalent of 1800 p.p.m., then $\frac{529{,}000 \text{ ml.}}{1{,}000{,}000} \times 100 = 952$ ml. per minute of ethylene equivalent are given off by the car.

Relation of epinasty to lemon coloring

Another experiment was set up in which 12 green lemons were placed in each of the 8 drums. The fruit for this test was selected as carefully as possible to get the same color, size, and type of lemons. The same amounts of ethylene and motor exhaust fumes were used as for the peas in the previous experiment. The lemons were left 10 days at 76° F. and then the drums were opened and the lemons examined for color.

The control fruit in no. 1 had colored very little.

The fruit in no. 2 was noticeably more colored than the control.

The fruit in no. 3 was not noticeably different from no. 2.

The fruit in no. 4 was slightly less colored than that in nos. 2 and 3.

The fruit in nos. 5, 6, 7, and 8 were progressively more colored, no. 8 almost fully colored.

These results indicate that the exhaust gases and ethylene are as effective in coloring lemons as they are in producing epinasty.

Another set of tests was run using different concentrations of ethylene. These data are given in table II.

TABLE II

Drum no.	Ethylene concentration	p.p.m.	Effect after 14 days
1 Control	None	......	Slight change in color
2	1 part to 40 million	0.025	No noticeable difference from control
3	1 part to 20 million	0.05	A little more color than control
4	1 part to 10 million	0.1	Slightly more color than in no. 3
5	1 part to 5 million	0.2	Quite well colored
6	1 part to 2.5 million	0.4	Almost fully colored
7	1 part to 1 million	1.0	Almost fully colored
8	1 part to 0.5 million	2.0	Almost fully colored

These data cover a range of concentrations much lower than those used previously but the fruit were left in for a longer time. There was no noticeable effect in 14 days by concentrations of less than 1 part in 20 million.

Figure 3 shows the effect of a similar range of concentrations of ethylene

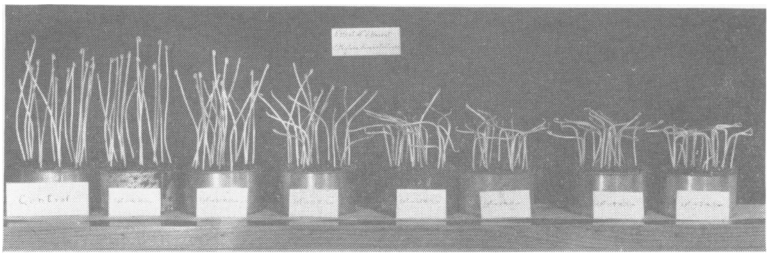

FIG. 3. Epinasty produced by different concentrations of ethylene on Alaska pea seedlings.

on peas under the same conditions as those used on the fruit. Table II and figure 3 show that there is a very close correlation between the concentrations necessary to cause bending in peas and coloring of lemons. Any ethylene concentration which is strong enough to cause epinasty or bending of the peas will cause an appreciable increase in the rate of coloring and maturing of lemons.

Ethylene equivalent content of lemon storage rooms

More or less green mold decay always develops among lemons during the storage season. We questioned whether the ethylene given off by this decay might be an important factor in the premature coloring and maturing of the fruit; also whether the exhaust gases given off by cars and trucks and entering the storage rooms through fresh air intakes might have that effect.

Since the storage rooms are kept at a temperature of 55–60° F. it was necessary in order to run the epinasty test to devise some means of maintaining the peas in the storage room atmosphere and at the same time to keep them dark and at a temperature of about 76° F. These requirements were met by making a container consisting of a can 6″ in diameter and 11″ high with a maze effect at the bottom and in the cover so that the air could come in around the bottom and go out at the top. This can was then equipped with a heater consisting of 2 10-watt intermediate base electric lights connected in series. Each light was covered with a collapsible metal tube to keep all light from the pea plants. This heater was placed in the bottom of the container and covered first by a piece of asbestos then by a small metal stand. On the stand was placed a can of peas previously grown in the can of peat moss and sand until 1 to 2 in. tall. The heater (shown in fig. 4) maintains the peas at very nearly an even temperature as long as the room temperature and air movement are the same. Different size lamps may be used in storages operated at other temperatures or thermostats may be employed.

This method of testing storage room air for the presence of ethylene appears to be quite satisfactory as a commercial method and is more accurate

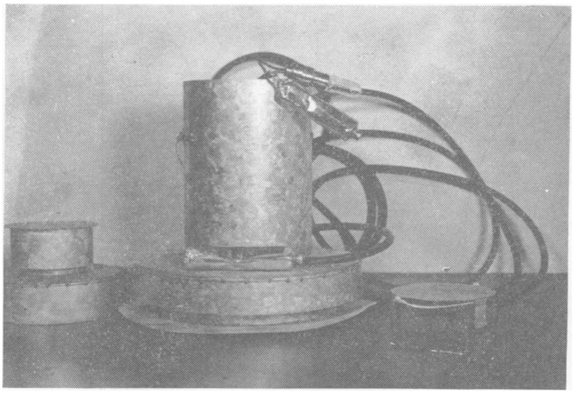

FIG. 4. Electrically heated dark chamber for growing peas in storage rooms.

than any of the usual chemical test methods. Peas can be grown continuously in the storage room; when one set is removed others are put in their place. Figure 5 shows pictures of peas grown in different lemon rooms.

Since figure 3 shows the effects of different known concentrations of ethylene, it can be used as a guide in estimating the concentration of ethylene in storage rooms.

The ethylene concentration of storage rooms may vary a great deal, depending on the amount of fresh air being brought in; the amount of fresh air brought in depending to a large extent upon the temperature and humidity outside as well as the refrigerating capacity available.

ETHYLENE EQUIVALENT PRODUCED BY DECAYING LEMONS

The rate of production of ethylene from one decaying lemon was roughly estimated by running air at the rate of 20 liters per hour through a jar containing a lemon decaying with *Penicillium digitatum* then through another jar containing a can of the pea seedlings. The effect on the peas indicates a concentration of ethylene of about 1 part to 7 or 8 million parts of air.

This means that enough ethylene or its equivalent is added to 20 liters of air flowing through the jars per hour to equal a concentration of 1 part ethylene in about 7.5 million parts of air or $\frac{1}{7{,}500{,}000} \times 20$ liters per hour or $\frac{480}{7{,}500{,}000}$ liters per day. This is equal to 0.064 ml. of ethylene per day or

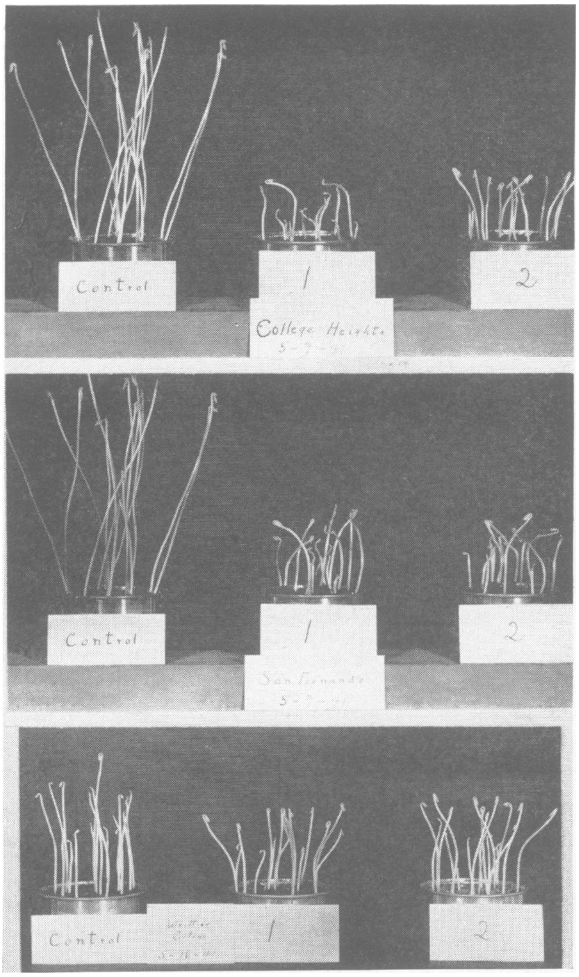

FIG. 5. Epinasty effects obtained on pea seedlings by ethylene in three different lemon storage rooms.

about 1 ml. in 15 days. While it has not been determined over how long a time this continues, it is considered probable that not much ethylene is given off after 15 days as the fruit is quite well decayed by that time.

SIGNIFICANCE OF ETHYLENE IN A STORAGE ROOM

BIALE and SHEPHERD (3) have shown that lemons give off CO_2 at the rate of 7 to 8 mg. per kilo of fruit per hour at 58° F. or an average of 180 mg. of CO_2 per kilo of fruit per day.

The work of GREEN *et al.* (**8**) shows that the heat given off by fruit and vegetables is in direct proportion to the CO_2 given off. They also found that within the limits of experimental error this relationship is the same as that for the combustion of sugar. The heat of combustion of sugar is 673,000 calories per mole, and since 1 mole of sugar gives off 6 moles of CO_2, one mg. of CO_2 is equal to 2.54 calories.

If, as BIALE has shown, a kilogram of lemons give off 180 mg. of CO_2 per day, then they also give off 180×2.54 calories or 457 calories of heat. One pound of lemons would give off 207 calories per day.

One car of lemons in storage contains 655 boxes of 50 lb. each or about 32,750 lb. Then 32,750 lb. $\times$ 207 or 6,779,250 calories of heat are given off per car per day at 59° F. Since 6,779,250 calories is the heat required to melt 187 lb. of ice, every 10.7 cars of stored fruit will require one ton of ice every 24 hours to remove the heat given off by the normal respiration.

BIALE and SHEPHERD (**3**) have shown that the respiration rate of normal lemons is approximately doubled by the respiratory gases given off by one lemon decaying with *Penicillium digitatum* when these gases are mixed with air and passed over the fruit at the rate of 3.6 liters per minute, a rate more than adequate to supply the oxygen for respiration of the fruit involved.

If it is assumed that this approaches a condition equal to that obtained for 1 per cent. green mold decay in a lemon storage room, then one additional ton of ice is going to be required by each 10.7 cars of lemons stored under these conditions.

Discussion

While the epinasty test has been known to be an indicator of the presence of ethylene, to the author's knowledge it has not been used to any extent commercially. Records of the California Fruit Growers Exchange indicate that the idea of using epinasty of tomatoes to measure ethylene in lemon storages existed as long as ten years ago. No way of growing tomato plants successfully in the low temperatures of the lemon storage was available at that time. In the present investigations it has served admirably as an accurate and simple test for ethylene.

One of the questions which will no doubt arise in the reader's mind is whether the carbon monoxide or some other of the automobile gases may not be responsible for the epinasty observed in experiments with these gases. CROCKER, ZIMMERMAN and HITCHCOCK (**4**) tested 38 different gases but found only five which produced epinastic effects and the minimum effective concentrations of those gases were as follows:

Ethylene	0.2 p.p.m. in air
Acetylene	250.0 p.p.m. in air
Propylene	1000.0 p.p.m. in air
Carbon monoxide	5000.0 p.p.m. in air

Butylene is not listed as affecting peas but is given as $\frac{1}{500,000}$ as effective as ethylene on tomatoes.

If there were as much acetylene as ethylene in the automobile exhaust gases it would be responsible for only $\frac{1}{1250}$ or about one-twelfth of one per cent. of the effect. KOBER and HAYHURST (**13**) list motor exhaust gases as containing about 9.3 per cent. carbon monoxide. This would be about 93,000 p.p.m. or about 52 times as much as ethylene; but since ethylene is 25,000 times as effective in producing epinasty, only about 0.2 of one per cent. of the effect would be due to carbon monoxide. It therefore becomes evident that carbon monoxide is of little consequence as compared to the ethylene in the automobile fumes.

The epinasty test for ethylene content in commercial storage rooms reported here is very simple and easily cared for after it is set up. It requires no technical skill except as is required to estimate the extent of epinasty in the pea plants in storage as compared with those kept as controls. In some locations in urban or other heavy traffic areas it may be difficult at times to find places to keep the control plants where they are not affected.

Two or three hours a week of a man's time is sufficient to keep tests going under most storage conditions.

Summary

1. This paper describes methods of using the epinasty reaction of pea seedlings for measuring small concentrations of ethylene in the exhaust fumes of automobile and in fruit storage rooms.

2. Data indicate a close relationship between the quantity of ethylene necessary to color lemons and that necessary to cause epinasty to pea seedlings.

3. The concentration of ethylene necessary to cause noticeable epinasty or lemon coloring has been found to be between 0.025 p.p.m. (1 part ethylene in 40 million) and 0.05 p.p.m. (1 part in 20 million) of air.

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