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The Occurrence in Pears of Metabolic Gases Other Than Carbon Dioxide

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TABLE OF CONTENTS

The Occurrence in Pears of Metabolic Gases Other Than Carbon Dioxide*

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INTRODUCTION

AT THE start of this investigation very little experimental data could be found relative to the evolution or presence in pears of volatile products other than carbon dioxide and acetaldehyde. Field observations by the authors and by Chace (1), however, have indicated that natural emanations from pears under certain conditions may accelerate ripening, and with this in mind work was undertaken to obtain evidence on the following inquiries:

I. Do pears evolve gases other than carbon dioxide and acetaldehyde, and if so, what is the nature of these gases?

II. If such gases do occur, what influence do they exert on the ripening and keeping qualities of pears?

In fruits other than the pear, there are data to the effect that volatile substances may be evolved which produce effects similar to those of ethylene or related gases. As long ago as 1910, Cousins (2) found that gaseous products from oranges hastened the ripening of bananas, and, more recently, Elmer (6) has reported that emanations from ripe apples inhibit normal sprout development in potatoes. Following the work of Elmer, similar results were announced by Huelin (10), who suggested that the active substance might be ethylene. Smith and Gane (15) and Kidd and West (12) have shown that gases produced by apples are similar to ethylene in that they increase the rate of respiration in apples and retard growth in germinating seeds. Very recently Gane (7) has shown by chemical methods that apples actually evolve ethylene.

BIOLOGICAL EVIDENCE

No specific chemical methods for the identification or determination of ethylene and related gases occurring in minute quantities were known to the authors at the beginning of this work. It has been shown by Harvey (8), Crocker (3), and others, however, that certain of these gases can be detected in very low concentrations by the peculiar physiological effects that they produce upon some plants. Mere traces of ethylene in the

*Note: This preliminary report is based upon data from a thesis submitted by Elmer Hansen to the Graduate Division, Oregon State Agricultural College, May 15, 1935.

Figure 1. Tomato plant after 18 hours in a container with normal atmosphere.

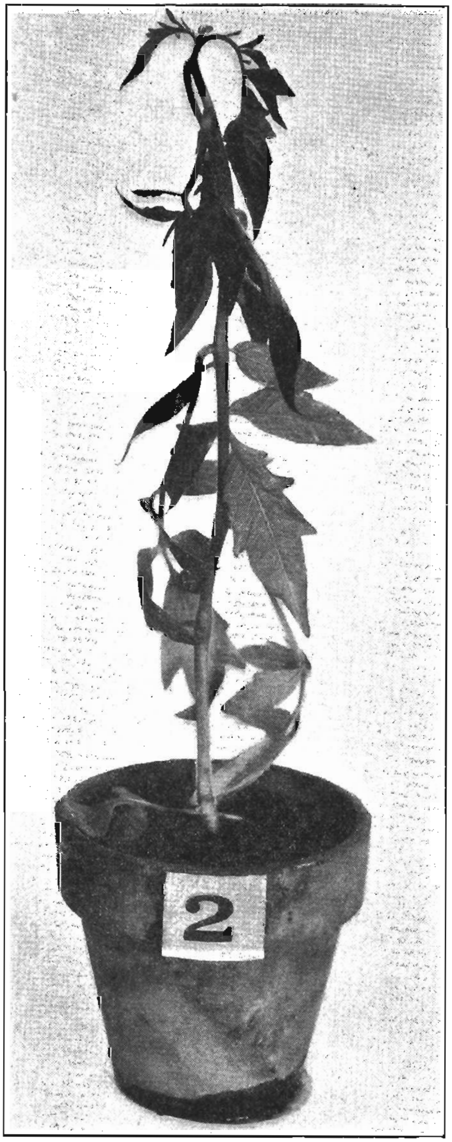

Figure 2. Tomato plant confined with pears for 18 hours.

Figure 3. Tomato plant after 18 hours of exposure to ethylene, 1 part to 1,000.

atmosphere, for instance, cause epinastic curvature in tomato leaves and retard the germination of certain kinds of seeds.

In the present experiments, either ten Anjou or ten Winter Nelis pears were enclosed in airtight containers with young tomato plants (6 to 10 inches in height) of the Bonny Best variety, or with germinating pea seeds. The containers used were five-gallon glass jars fitted with airtight lids and inlet and outlet tubes. A false bottom of galvanized mesh wire was used in each jar to support the fruit and to permit the use of a potassium-hydroxide solution underneath the fruit to absorb the carbon dioxide evolved. Prior to each experiment the jars were filled with water to displace any foreign gas that might have been present. After the jars were sealed, the lids were tested for leaks, and twice daily fresh air was admitted by momentarily opening the inlet tubes.

The experiments were conducted with fruit being held at 65° Fahrenheit as well as with unripened fruit held at 31° Fahrenheit in cold storage. In the case of the fruit in cold storage, the emanations from approximately 13 kilograms of pears were collected into a separate jar over a period of twelve hours and were later released at room temperature into a jar containing a tomato plant. In all cases check experiments were run, and jars were also set up wherein tomato plants and germinating seeds were subjected to ethylene in various concentrations.

The results of these experiments show that pears in closed containers produce effects on germinating pea seeds and on tomato plants that are indistinguishable in outward appearance from those caused by ethylene. In the case of tomato plants, symptoms of leaf curvature (Figure 2) usually appeared in about three hours, and at the expiration of eighteen hours marked epinasty had developed. Ethylene in concentrations of 1 part in 1,000 or less (Figure 3) produced the effect in approximately the same length of time. Epinasty was induced by the emanations from pears in various stages of ripeness held at 65° Fahrenheit as well as by the emanations of unripened pears held in cold storage. Peeled pears as well as peelings alone were effective, but pear tissue that had been rendered inactive by freezing or by treatment with chloroform gave no response.

The evolution of the substance responsible for the foregoing symptoms appears to be associated with the respiratory activities of the fruit. This is indicated by the fact that the effects occurred only when the fruit used was respiring normally. They did not occur in the case of fruit that had reached a state of senescence wherein respiration was known practically to have ceased.

CHEMICAL EVIDENCE

Crocker, Zimmerman, and Hitchcock (4), working with a large number of gases, concluded that only ethylene, acetylene, propylene, carbon monoxide, and butylene induce epinasty in tomato plants, and they also determined the minimum amount of each of these gases necessary to produce epinastic effects. The minimum concentrations needed to produce the effect are given by these authors as follows:

Ethylene	1 part in 10,000,000
Acetylene	1 part in 20,000
Propylene	1 part in 20,000
Carbon monoxide	1 part in 2,000
Butylene	1 part in 200

Since epinasty was induced upon enclosing tomato plants with pears, it is fairly safe to assume that the fruit used evolved one or more of the foregoing gases in sufficient quantities to have caused this effect. Considering the great difference in minimum effective concentrations between certain of these gases, a quantitative determination of the emanations inducing epinasty should give some indication as to which of the five gases is present in amounts high enough to affect tomato plants in this manner.

In attempting to measure the gas evolved, the method of Kinnicutt and Sanford (14) was used as for the determination of carbon monoxide. It was thought that this method was applicable since preliminary tests had shown that the effectiveness of the gases from pears in causing epinasty was reduced by being passed through iodine pentoxide heated to 150° C., and that the iodine liberated could be detected by absorption in chloroform. Preliminary trials had also shown that although acetaldehyde was present in the air stream, the concentration was not great enough to cause any interference with the test. By using a large quantity of fruit, sufficient iodine could be liberated so that titration with .005 N sodium thiosulfate was possible after absorption in a solution of potassium iodide.

Accordingly a number of determinations were made, using 32 kilograms of semiripe Anjou pears. The titration values obtained were calculated as each of the five gases—carbon monoxide, ethylene and acetylene on the basis of complete oxidation, and propylene and butylene on the basis of partial oxidation. The values obtained for each gas were then compared with the value for the minimum amount known to cause epinasty.

Calculated as ethylene, the gas reducing iodine pentoxide was found to be present in the air stream, drawn from 32 kilograms of fruit at the rate of 9 liters per hour, in a concentration of approximately 1 part by volume in 70,000 of air, an amount well within the range known to cause epinasty in tomato leaves. The gas calculated as carbon monoxide, acetylene, propylene, or butylene, however, was found to be present in amounts far below the minimum effective concentrations of these gases. The indications are, therefore, that ethylene is the only one of the gases named that could have been present in concentrations sufficient to have caused the effects noted.

Further evidence that ethylene is evolved by pears was obtained by determining the solubility of the emanations in a solution of mercuric nitrate. According to Hoffman and Sands (9) ethylene absorbed in this manner can be liberated in gaseous form by hydrochloric acid. By this means Curme (5) obtained pure ethylene from inert gaseous mixtures, and Treadwell and Hall (16) separate ethylene from acetylene. If the gas from pears, then, induced epinasty after being subjected to this treatment, strong evidence would be offered that ethylene was present. Accordingly, the emanations from approximately 13 kilograms of ripe Anjou pears were passed through 50 milliliters of 20-per-cent mercuric nitrate solution (in 2N nitric acid) contained in a Truog absorption tower. After twelve hours the solution was transferred to a wash bottle that was connected with a jar containing a tomato plant. The air in the jar was partly exhausted, dilute hydrochloric acid was added to the solution, and the gases evolved were drawn by the partial vacuum into the atmosphere surrounding the tomato plant. Blánk determinations were run to make sure that gases responsible for epinasty were not being evolved by the solution alone.

This experiment was repeated a number of times. In each case marked epinasty occurred in approximately six hours. The effects were identical in appearance to those produced by ethylene and by the untreated emanations from pears.

While this method is effective in separating ethylene from acetylene, it is not so certain that propylene or butylene may not have been absorbed under the same conditions. That ethylene is the gas absorbed by the mercury salt solution, however, is indicated by the fact that the emanations from pears were still effective in causing epinasty after having been passed through 87-per-cent sulfuric acid, which removes both propylene and butylene (17) but in which ethylene is not appreciably soluble.

It is fully realized that these experiments do not prove beyond doubt that ethylene is evolved by pears, nor that ethylene is the only gas responsible for the effects noted. That this is the case, however, is strongly indicated. In view of the evidence obtained, it appears that the gas must be ethylene, since the only other gases known to produce epinasty are carbon monoxide, acetylene, propylene, and butylene, which apparently are not present in pears, at least in sufficiently large amounts to induce these effects. Moreover, the elimination of acetylene, propylene, and butylene by specific absorption, and the relatively high concentration of carbon monoxide necessary to induce epinasty, indicate that ethylene is the active substance. Further, very strong evidence that the gas is ethylene is indicated by the fact that the emanations from pears when absorbed by mercuric nitrate solution produced epinasty after being released in gaseous form by hydrochloric acid. Also, the evidence of the occurrence of ethylene in apples (7) suggests that this gas may also be present in pears, since the metabolic processes of the two fruits are similar in many respects.

INFLUENCE OF THE GAS PRODUCED BY PEARS UPON THE RATE OF RIPENING

As ethylene is known to accelerate ripening in certain fruits, it is logical to suppose that if this gas is evolved by pears, the emanations from one lot of pears might influence the rate of ripening of other lots exposed to the same atmospheric conditions. Ripe pears, for example, might be expected to accelerate ripening in unripened pears when the two are enclosed in the same chamber or container. This is all the more likely as similar response has been reported for apples and bananas (12), and as it appears that the amount of ethylene evolved by pears is comparable to that reported as influencing the ripening of some fruits (13).

That the gas evolved by pears does have a very pronounced influence upon the rate of ripening and respiration of immature and newly picked fruit has been shown by preliminary results of experiments now in progress. Bartlett, Comice, and Anjou pears picked in immature stages have ripened in 8 to 15 days when subjected to the influence of several ripe pears in closed containers. Duplicate lots, however, that were not subjected to the influence of ripe fruit, but kept under constant ventilation with fresh air, either failed to ripen or were markedly delayed beyond the time required for treated fruit.

So far as can be determined from experimental data now available, the effects of the gas influencing ripening are confined to newly picked fruit or to fruit that has been held in storage for only a short period of time. A series of experiments carried on with cold-storage pears during the period from December to March, 1934-35, have given only inconclusive results. The ripening rate of these pears was not influenced by the presence of ripe fruit, nor was it affected by ethylene artificially applied. Fruit in aerated containers ripened at approximately the same rate as the fruit subjected to the emanations from pears or to treatment with pure ethylene. In the case of the unaerated containers the carbon dioxide evolved was kept at a concentration below that which could be detected with a Hempel apparatus by the use of a potassium-hydroxide solution, and atmospheric concentration of oxygen was maintained by the addition of pure oxygen from a constant-water-level siphon. It is therefore not likely that ripening was retarded in the closed containers by an accumulation of carbon dioxide or by a deficiency of oxygen.

The marked difference in results obtained between newly picked and cold-storage fruit can probably be explained on the basis that the effects of ethylene are confined to a preripening period that apparently is initiated during maturation on the tree and is completed early in storage. The indications are that actual ripening processes—i.e., the softening of the flesh—and the development of flavor and juiciness do not take place until the termination of this period, and furthermore that the presence of ethylene in the atmosphere surrounding the fruit is without effect beyond this period.

The phenomenon of a preripening period is apparently not peculiar to pears but occurs in other fruits as well. Kidd and West (11) first noted a similar period in apples and designated it as the "climacteric". They have subsequently reported (12) that a climacteric also occurs in bananas and tomatoes and that the gaseous products from ripening apples are without effect on post-climacteric apples and bananas. If a similar phenomenon occurs in pears, as is now indicated, it is not surprising that such contrasting results were obtained with the fruit used in the present experiments. Furthermore, the wide diversity of results that have been obtained in the use of ethylene as a ripening agent can be explained at least partly on the same basis.

SUMMARY

1. The presence of pears in closed containers has induced epinasty in tomato plants and has retarded the germination of certain kinds of seeds.

2. Emanation of the substance responsible for these effects took place in ripe pears at room temperature and in unripened pears in cold storage.

3. Chemical evidence indicates that ethylene is responsible for the effects noted.

4. Ripening tests show that emanations from pears have a pronounced influence in hastening the ripening rate of newly picked fruit. No response could be obtained, however, from fruit that had been held in cold storage for a short length of time.

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*Note: The publication by F. E. Denny and Lawrence P. Miller, entitled *Production of Ethylene by Plant Tissue as Indicated by Epinastic Responses of Leaves*, Boyce-Thompson Inst. Contrib. vol. 7 no. 2: 97-102, 1935, came to the attention of the authors after this paper had been prepared for publication.

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