

# RELATION OF THE PRODUCTION OF AN ACTIVE EMANATION TO RESPIRATION IN THE AVOCADO FRUIT

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(WITH SEVEN FIGURES)

## Introduction

The production of physiologically active emanations by plant tissue in general, and especially by ripe fruit, has been demonstrated by epinastic responses of tomato and potato leaves, by inhibition of seed germination and potato sprouting, by the triple response of etiolated legume seedlings, and by effects on fruit respiration and softening. In all cases which subsequently have been investigated chemically, the active substance has been shown to be ethylene. This is now so well established and generally accepted that demonstration of the activity of an emanation by any of the above methods is a very strong indication that ethylene is among the gases evolved [(18, 19, 20, 21) and review by BIALE and SHEPHERD (4)].

A number of workers have found that the production of ethylene by various fruits is associated with the increase in rate of carbon dioxide output which is known as the climacteric rise in fruit respiration. HANSEN (7, 8) and HANSEN and HARTMAN (9) found that the peak of ethylene production coincided closely with the climacteric peak in the pear. KIDD and WEST (11) report that the development of the substance in apple vapor which inhibits germination of pea and mustard seeds coincides with the climacteric rise. NELSON (14, 15) found that several varieties of apples show a pattern of varying ethylene content throughout storage life analogous to the course of carbon dioxide evolution. Subsequently (17) he compared the evolution of carbon dioxide and ethylene by the McIntosh variety. In this study it appeared that the peak of ethylene production occurred somewhat later than the peak of carbon dioxide output. On the other hand, for the banana (14, 15, 16) he found that the highest rate of ethylene evolution comes before the peak of carbon dioxide production, with a secondary peak afterward; evidence was presented that ethylene is consumed during the peak of carbon dioxide output.

DENNY and MILLER (6) obtained a positive epinastic response with young potato plants subjected to the emanations of avocado fruit, and recent studies on the respiration of the avocado (2, 3, 22) have shown that this fruit has a well-marked climacteric rise. It therefore became of interest to study the relation between production of active emanations and the course of respiration.

## Materials and methods

Etiolated legume seedlings are markedly affected by minute concentrations of ethylene gas, showing the "triple response" which is defined by

KNIGHT and CROCKER (12) as a "change of negative geotropism to diageotropism, increased growth in thickness, and reduced rate of growth in length." That the response of the pea epicotyl varies with the concentration of ethylene, giving well-defined qualitative and quantitative responses at different levels, was shown by NELJUBOW (13). ROHRBAUGH (18) has shown that the etiolated Alaska pea responds to as little as 0.05 parts per million of ethylene in air, and this has been confirmed by work in progress in this laboratory. Accordingly, young etiolated pea seedlings of this variety were chosen as test plants for the present study; uniform lots can be produced easily at any time, and their sensitivity is about equal to potato or tomato leaf epinasty. The maximum response is produced by five to ten parts per million of ethylene (fig. 1).

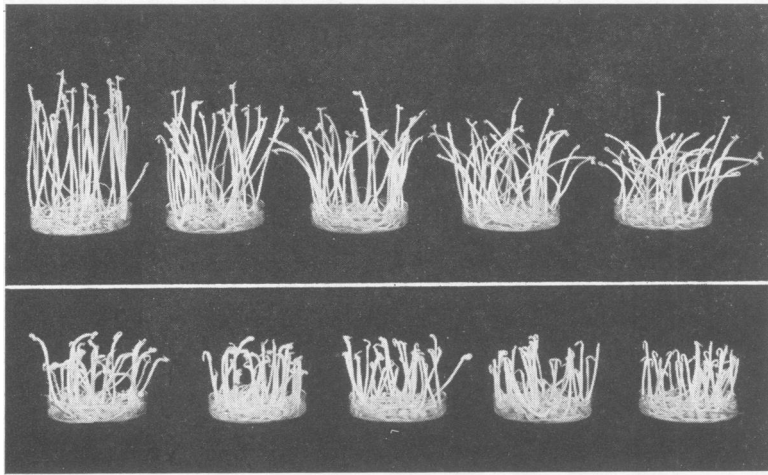


FIG. 1. The response of etiolated pea seedlings at 25° C. to known concentrations of ethylene gas in air. Top (from left): Control, 0.05, 0.10, 0.15, and 0.25 parts per million. Bottom: 0.50, 1.0, 2.5, 5.0, and 10.0 parts per million.

Test sets of peas were prepared by the following series of operations, all conducted in a room maintained at 25° C.: Lots of seed were soaked for seven hours in distilled water and then placed on wet filter paper in ten-centimeter Petri dishes, fifty seeds per dish. After two days, they were examined and reduced to the most vigorous thirty-five seedlings per dish. At this time the dish covers were removed, and the peas were watered with a saturated solution of calcium sulphate and placed in a humid atmosphere in the dark. On the sixth day, the shoots were about four centimeters high and the roots well developed. At this time they were ready for use, but if not used at once they could be kept in the dark at 5° C. until needed (as long as two weeks), resuming normal growth on return to 25°. Saturated calcium sulphate solution was used for watering the seedlings because it provided an easily prepared medium of constant composition which gave significantly better results than distilled water.

The test for active emanation was conducted by passing the air stream from a jar of fruit into the 25° room, through a water bubbler to equalize temperature and humidity, and through a large metal can containing two dishes of pea seedlings. The growth of the test plants relative to the controls was not affected by the original temperature of the air stream, by change in the rate of flow of air (200 or 350 ml. per minute), or by the reduced oxygen content of a modified atmosphere. The duration of exposure of each set of test plants was two days, after which the peas were photographed and a fresh set placed in series with the fruit, the photograph being marked with the date of removal of the set shown. The fruit was handled and respiration determined by methods previously described (2). Three storage temperatures were used—5°, 15°, and 25° C. The air stream was not connected to the pea containers during fruit respiration determinations (four hours per day).

## Results

### PRODUCTION OF ACTIVE EMANATION IN RELATION TO CARBON DIOXIDE EVOLUTION

RESULTS UNDER NORMAL ATMOSPHERE.—In a preliminary experiment it was found that Fuerte avocados at 15° gave off a gas which, during the period of respiratory rise, induced the triple response in etiolated pea seedlings. A more detailed study of this behavior was undertaken with fruit stored at different temperatures. Twenty freshly picked mature fruits (four to five kilos fresh weight) of the Fuerte variety were stored in each jar, with three jars for the first experiment and two for the second, one jar being placed at each temperature studied. The fruit in the 15° and 25° chambers was subjected to an air flow of 350 ml. per minute, while that in the 5° room was at 200 ml. per minute. The first carbon dioxide determination was made the day after picking. The results of these experiments are shown in figure 2.

It readily can be seen that the agreement in respiratory trend of the fruit at corresponding temperatures in the two experiments is remarkably close. In both cases the respiratory magnitudes were the same, and the time lapse between minimum and maximum respiratory activity was three days at 25° and five days at 15°, although the rise started one day sooner in the second experiment. The rates of carbon dioxide evolution, however, are markedly different at the two temperatures, and the slopes of the ascending curves are twice as great at 25° as at 15°. At the peak of the climacteric rise, the temperature coefficient ( $Q_{10}$ ) for the range of 15° to 25° was 1.84 in the first experiment and 1.77 in the second.

In the first experiment, the fruit at 25° (jar 31) did not produce enough of the active emanations through the second day in storage (March 24) to cause any response in the first set of test plants. One day later, an initial response was shown by a definite decrease in length growth of the second set. On the fourth day (March 26) the concentration of emanation had increased

sufficiently to cause marked thickening and horizontal growth of the second set of peas. At that time the fruit at 15° and 5° still gave no response, as shown in figure 3. The first evidence of a response from fruit at 15° (jar 25A) was observed in the third set on the fifth day (March 27), coinciding with the first stage of the rise in carbon dioxide evolution. This date marked the carbon dioxide peak for the fruit at 25°, and its maximum pea response was shown by this third set. On the eighth day (March 30), the respiration rate at 15° was at its maximum, as was the pea response (fig. 4, the fourth test set). On the other hand, the fruit at 25° had already passed its climacteric, and the pea response was significantly diminished. In the second

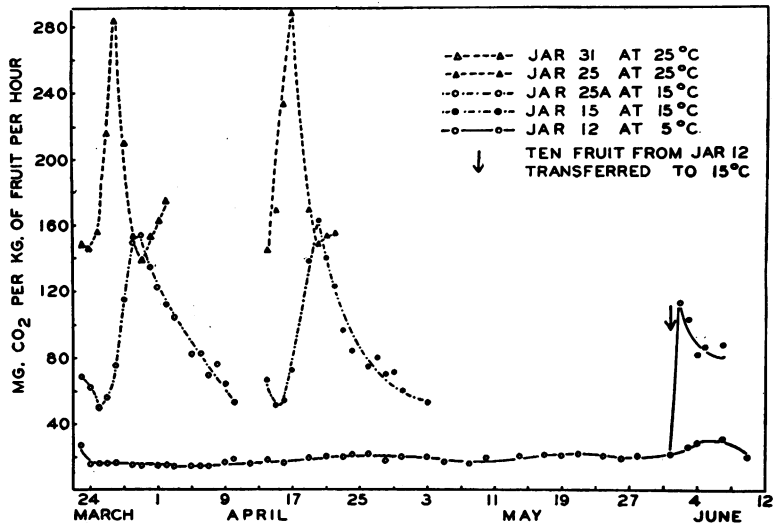


FIG. 2. Carbon dioxide evolution by Fuerte avocados stored in air at different temperatures.

experiment the same relationship between emanations and respiration was shown.

The analysis of fruit behavior at 5° presents some difficulties because of the very much reduced respiration rates. From a superficial examination of the curve, it would seem that the climacteric peak took place during the period of June 4 to June 7. This, however, is not in conformity with previously discovered facts and with new evidence to be introduced here. The climacteric rise must have taken place prior to June 1, because the fruit transferred to 15° on that day shows a descending curve of carbon dioxide evolution, with a maximum observed value much lower than that expected for this temperature. It was shown in a previous paper (3) that post-climacteric fruit will show this sharply descending curve when moved to a higher temperature, while preclimacteric fruit gives a typical increase to a maximum followed by a decrease in respiration. This contention will be supported further by an examination of the pea responses. Although at 5° (jar 12) the effect of avocado emanations on the growth of peas is less

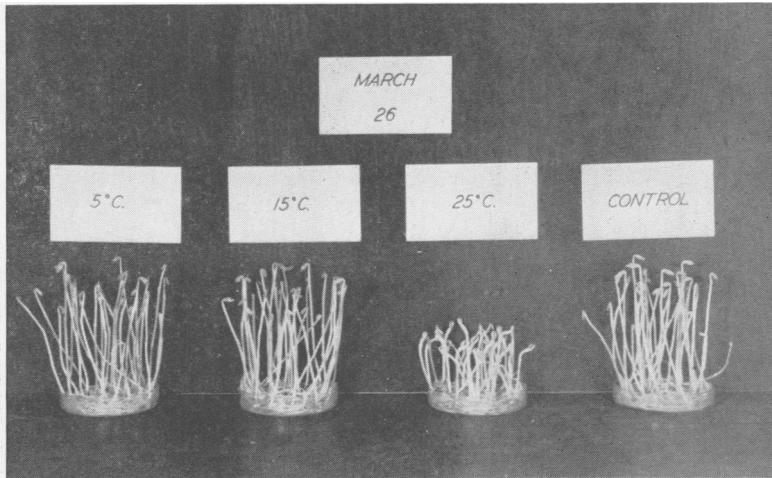


FIG. 3. The response of etiolated pea seedlings at 25° C. to emanations from Fuerte avocados stored in air at the temperatures indicated.

sharply defined, as early as April 9 the peas appeared slightly shorter than the controls. It was not until almost mid-May that marked horizontal growth was first noted. A maximum pea response was observed for the period from May 18 through May 22 (fig. 5), after which the response decreased gradually. A definite, though weaker, response persisted to the end of the experiment. The behavior of the fruit at 5° suggests that, under these conditions, the production of an active emanation may be a better index for the physiological changes characteristic of the climacteric than the rate of carbon dioxide evolution.

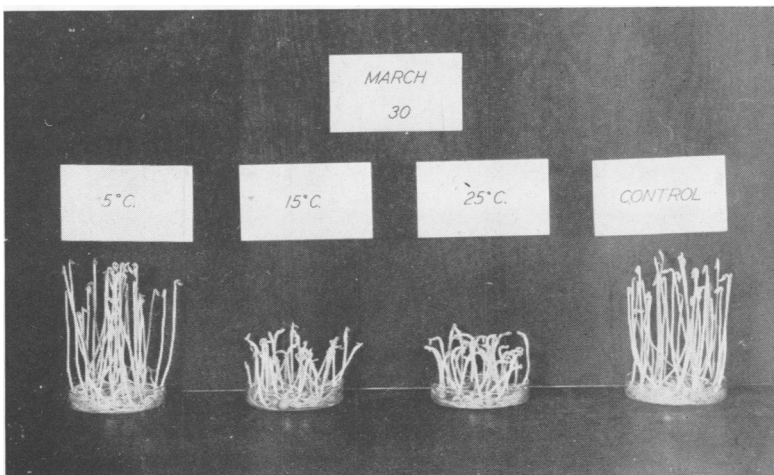


FIG. 4. The response of etiolated pea seedlings at 25° C. to emanations from Fuerte avocados stored in air at the temperatures indicated.

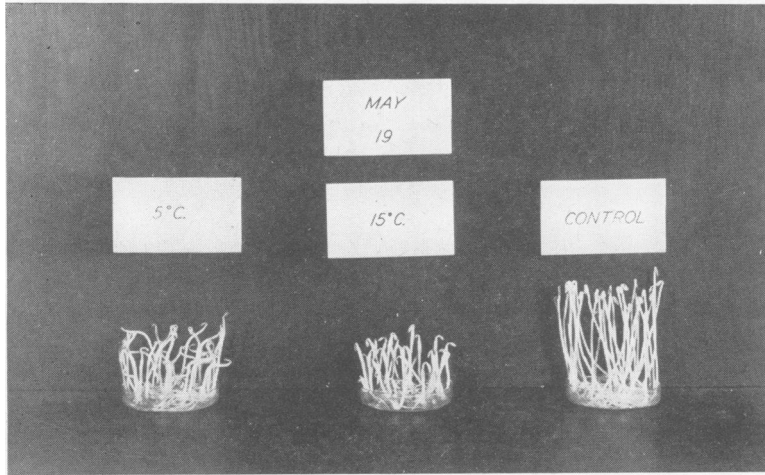


FIG. 5. The response of etiolated pea seedlings at 25° C. to emanations from Fuerte avocados stored at 5° C. in air and at 15° C. in a modified atmosphere (2.5 per cent. oxygen in nitrogen).

The activity of avocado fruit emanation was further verified by tomato leaf epinasty in one instance. On April 7, one avocado from jar 25A was transferred to 25° and placed in a jar with a tomato plant having four well-developed leaves. The carbon dioxide given off was absorbed in potassium hydroxide solution, and the oxygen was replenished from a bottle under the pressure of a constant water level siphon. The strong response produced in 24 hours is shown in figure 6.

An experiment similar to those described for the Fuerte avocado was performed with Nabal, one of the leading summer varieties in California.

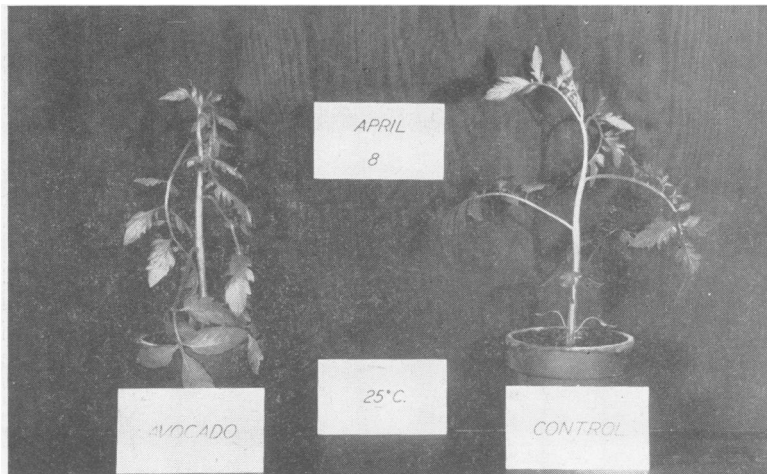


FIG. 6. Tomato leaf epinasty induced by emanation from a Fuerte avocado fruit.

Nine freshly picked mature fruits (four to five kilos fresh weight) were used for each container. In this case the fruit was placed at 15° and 25° only. The trend of respiration was very similar to that of the Fuerte, although the respiratory activity was somewhat lower, particularly before the climacteric rise. At 15° the minimum carbon dioxide values were about fifty per cent. less than in the case of Fuerte, and it took twice as long for the maximum to be attained. The reaction of the peas showed, however, that the trend of fruit emanation was essentially the same for the Nabal avocados as for the winter variety, with the maximum response coinciding with the highest rate of carbon dioxide production.

EFFECTS OF LOW ATMOSPHERIC OXYGEN.—In experiments with different oxygen tensions in relation to respiration and storage (3), it was found that

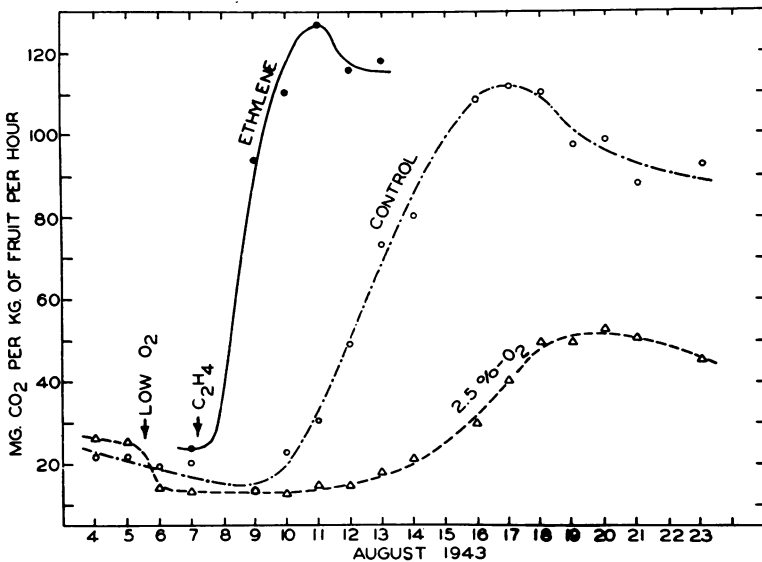


FIG. 7. Carbon dioxide evolution by Nabal avocados at 15° C. under treatment with ethylene gas (200 p.p.m.), with a modified atmosphere (2.5 per cent. oxygen in nitrogen), and in air (control). Treatments started at the times indicated by arrows.

the climacteric rise can be delayed and its magnitude reduced by subjecting the fruit to a reduced oxygen content of the atmosphere. The question arose, therefore, as to what effect this might have on the production of active emanations. It can be seen from figure 7 that the peak in respiration of Nabal fruit at 15° under 2.5 per cent. oxygen (in nitrogen) was reached four days later than in the control, and its value was decreased by more than 50 per cent. On August 13 the peas subjected to the air stream from the control fruit showed horizontal growth, while those connected to the fruit under low oxygen were only slightly reduced in length. Maximum pea response was reached by August 16 for the control and by August 20 to 23 for the modified atmosphere set. These dates correspond with maximum respiratory activity. Results obtained earlier with Fuerte under the

same conditions were similar, the reduction in carbon dioxide output being even more striking. Although respiration is markedly reduced, sufficient active emanation is produced under low oxygen to give the maximum pea response (fig. 5, 15°).

#### TREATMENT OF AVOCADOS WITH ETHYLENE GAS

Many plant materials are known to show a physiological response to applied atmospheric ethylene. This particularly applies to mature fruit in its pre-climacteric state, ethylene causing more rapid onset of the climacteric rise and the related ripening processes (9). The treatment of avocados has been considered by other workers; HARVEY (10) and ADRIANO *et al.* (1) treated fruits of the Baldwin, Cardinal, Lyon, and Pollock varieties with 1000 parts per million of ethylene in air, securing softening and good flavor in two to three days. On the other hand, CHACE and CHURCH (5) treated avocados of the Blakeman, Challenge, Colorado, and Solano varieties at high temperature with 2000 parts per million with unsatisfactory results. The latter, however, must be discounted as the fruit studied was not commercially mature. In none of the cited cases was the respiration rate followed in conjunction with ethylene treatment.

Nabal fruits from the same lot as used in the emanation study above were treated with 200 parts per million of ethylene gas at 15°, and the effect of the treatment on respiration was observed. During twenty hours a day, the fruit was kept in a closed system in which carbon dioxide was absorbed by an excess of potassium hydroxide solution, and oxygen was added from a bottle under pressure of a constant water level siphon. Ethylene was added to the fruit jar from a gas burette (2 ml. per 10 liters of free space). During the remaining four hours, the potassium hydroxide was removed, and carbon dioxide-free air was passed through the jar at 350 ml. per minute for respiration determination. After this period, the potassium hydroxide and ethylene were renewed. The control jar was subjected to an air stream throughout the experiment. In a subsequent experiment two controls were used, one in a closed system as described above and the other subjected to an air stream. No significant difference due to confinement in the closed system was observed.

The result of ethylene treatment is seen in figure 7. The climacteric rise was initiated immediately after ethylene was applied, and the respiration rose rapidly, reaching its peak in four days. The fruit was found to be fully softened on the following day. On the other hand, the control fruit started its rise four days later, required five to six days to reach the peak, and softening was correspondingly delayed. Furthermore, the control lot was variable in softening time, while the ethylene-treated fruit softened uniformly and was of somewhat better quality.

#### Summary

The carbon dioxide evolution of Fuerte and Nabal avocados was studied at three different temperatures. At 15° and 25° C. a very pronounced climacteric rise was observed, while at 5° C. there was no well-defined increase.



Associated with the climacteric rise was the production of an active emanation, presumably ethylene, as shown by the triple response of etiolated pea seedlings. Although there was no obvious climacteric rise at 5°, a cycle of emanation production was observed, after which the fruit showed a post-climacteric carbon dioxide response when transferred to a higher temperature.

Treatment of pre-climacteric fruit with ethylene caused an earlier onset of the climacteric rise and more uniform softening.

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