

EFFECT OF ETHYLENE AND CERTAIN METABOLIC GASES UPON RESPIRATION AND RIPENING OF PEARS BEFORE AND AFTER COLD STORAGE

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(WITH FIVE FIGURES)

Introduction

Previous work by the writers (6) has shown that the ripening rate of newly picked pears can be markedly stimulated by ethylene or gases of similar properties naturally evolved, but fruit treated after being held for prolonged periods of cold storage is not similarly affected. As a tentative explanation of these differences, it was suggested that the effects of ethylene are confined to a pre-ripening period, and later ripening changes apparently are unaffected by the presence of the gas in the atmosphere surrounding the fruit. These observations are in agreement with those previously reported by KIDD and WEST (1) for apples.

The present investigation was undertaken to further study the effects of naturally occurring ethylene upon the ripening processes in pears and to more definitely determine if there is a stage in the life of the fruit when treatment with this gas is most effective.

Material and Methods

The varieties of fruits used in these experiments were Bartlett, Bosc, Comice, and Anjou pears. With the exception of the first mentioned variety, these were grown in the Medford (Oregon) district. Fruit intended for treatment immediately after picking was gathered at approximately ten-day intervals, beginning a month or more prior to and extending considerably beyond the regular commercial harvest season. Fruit intended for storage studies was picked at one date when the proper picking stage for the variety had been reached.

Immediately after picking or removal from storage, a pressure test was taken of each lot, which was then subdivided into two duplicate lots of 10 to 15 pears each. These were weighed, placed in five-gallon glass jars provided with air-tight lids, fitted with inlet and outlet tubes.

To prevent any accumulation of gases that might be effective in stimulating ripening of fruit within the containers, the untreated lots were provided with constant aeration in the following manner: An air line with an inlet to a fresh air supply was installed in the ripening room. The jars containing the untreated samples were connected to outlets on the air line and continuous ventilation was provided by means of a vacuum pump operating

on the reverse end. The incoming air before passing over the fruit was brought to room temperature and conditioned for humidity by passing through a five-foot length of pipe and finally bubbling through one-half inch of water maintained at room temperature.

The treated fruit was supported in the jars on a false bottom, permitting the use of a 15 per cent. KOH solution beneath to absorb the carbon dioxide evolved and to maintain a humidity comparable to that in the ventilated jars. Atmospheric concentration of oxygen was maintained by the addition of pure oxygen from a constant water level siphon. In those lots treated with ethylene artificially applied, the concentration of gas used, in all cases, was one part by volume to 1000 parts of air. In the tests with naturally evolved gases, three or four ripe pears were enclosed in the jar together with the fruit to be ripened. The fruit was kept in the containers continuously during the time of treatment except for a period of one to two hours daily when removed to determine the rate of respiration. In the cases where ethylene was applied, the gas was replenished after each removal of the fruit from the jars. The KOH solutions were renewed often enough to assure efficient absorption of carbon dioxide.

Respiration determinations were conducted in a room maintained at a temperature of 65° F. Although thermograph records showed in some cases a variation of two to three degrees, this was not considered to have materially affected the differences in rate of respiration observed between treated and untreated lots, since both were always subject to the same temperature changes. The method of HARDING, MANEY, and PLAGGE (7), with slight modifications, was used for carbon dioxide determinations. The carbon dioxide was absorbed in 0.15 N barium hydroxide and titrated directly with the N/10 hydrochloric acid, using thymolphthalein as indicator. Determinations were made daily for a period of seven to nine days with the storage series, but with newly-picked fruit this period was considerably extended because of the longer period of time required for ripening. At the end of the experiment, loss in weight for each lot was determined.

The rate of ripening of treated and untreated lots was determined by the comparative softness of the flesh as indicated by the Oregon pressure tester, and by the number of days required for the fruit to attain an edible condition. With the newly picked fruit, where carbon dioxide determinations were extended in some cases over a period of thirty days or more, the number of days required for ripening only was used as an index of ripening rate. With the storage series, a pressure test was made on one-half of both treated and untreated lots at the end of nine days and the remainder kept to determine the number of days required for ripening. If the fruit was ripe before the expiration of nine days, the entire sample was used for a pressure test.

Experimental results

I. EFFECT OF NATURALLY EVOLVED GASES AND OF ETHYLENE ARTIFICIALLY APPLIED UPON RESPIRATION AND RIPENING OF NEWLY-PICKED FRUIT

SELECTION OF SAMPLES AND TREATMENT.—The samples of each variety used in the experiments with newly-picked fruit were selected from single trees and represented the average size of the fruit at the time of gathering. The first two pickings of Bartlett were made approximately two months prior to the commercial harvest season, but because of the small size and undeveloped condition of these fruits no treated lots were included. The data are presented, however, to show the trend in respiration at this early period in the season. A sample of the third picking was treated with ethylene gas, since no ripe pears were available at that time, but all subsequent lots of newly-picked fruit to be treated were inclosed with ripe pears as indicated in the general methods.

TABLE I

EFFECT OF RIPE FRUIT UPON THE RIPENING OF NEWLY-PICKED BARTLETT, ANJOU, AND COMICE PEARS

VARIETY	DATE PICKED	DAYS REQUIRED FOR RIPENING		DIFFERENCE IN DAYS REQUIRED FOR RIPENING
		TREATED	UNTREATED	
Bartlett	7-22*	8	20	12
	8-20	8	14	6
	9-4	7	12	5
	9-17	6	8	2
Comice	8-16	20	†
	8-30	15	†
	9-20	11	30	19
	10-8	8	15	7
Anjou	8-16	18	†
	8-30	12	†
	9-16	12	35	23
	10-8	8	15	7

* Treated with ethylene.

† Failed to ripen in 30 days.

EFFECT UPON RATE OF RIPENING

That the gas produced by ripe pears does have a very pronounced effect upon the ripening of newly-picked fruit is evident from these studies. As shown in table I, the treated lots, subjected to the influence of several ripe pears in closed containers, ripened in comparatively short periods of time.

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 ripening of the treated fruit.

But it is clearly evident from the results obtained that while the period required for ripening can be significantly reduced by these naturally occurring emanations, the magnitude of the decrease obtained depends upon the degree of maturity of the fruit at the time of picking. Thus, with an early picking of immature Bartlett, there was a difference of 12 days between the ripening time of treated and untreated lots. With post-mature fruit, however, the ripening period of the treated lot was only two days less than that of the untreated. Similar results were observed with Anjou and Comice pears. These varieties respond to ethylene over a longer period, however, than do Bartlett. Reference to table I shows that while both of these varieties were markedly affected in rate of ripening by the emanations from ripe pears, the greatest response was obtained in those lots picked early in the season. Thus, the treated fruit of the first pickings ripened in 15 to 18 days, while the untreated lots failed to ripen in 30 days and the condition of the fruit at that time was such as to indicate that ripening would be delayed indefinitely. Later pickings showed decreasingly smaller differences in the ripening time between treated and untreated lots. Post-mature samples, however, picked three weeks after the commercial season still showed seven days' difference between treated and untreated lots.

EFFECT UPON RESPIRATION

The increase in the rate of ripening of the treated fruit was accompanied by a marked and rapid increase in respiration (figs. 1, 2, 3). The gases evolved by ripe pears inclosed with the unripened fruit had the same effect in stimulating respiration as did ethylene added to the containers. In the

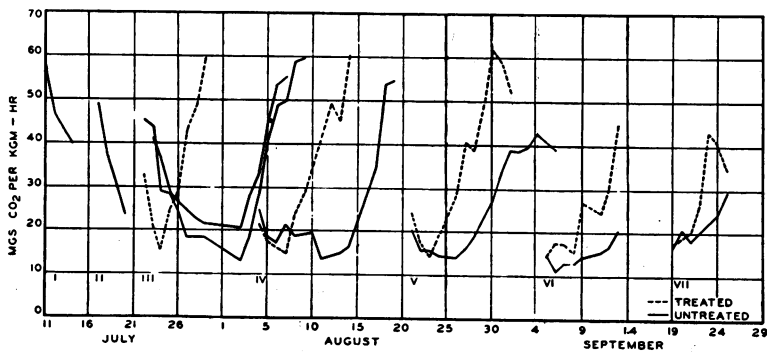


FIG. 1. Effect of gases produced by ripe pears upon the respiration of Bartlett pears picked at different stages of maturity.

untreated lots no similar increase in respiration occurred until after a delayed period of time. The length of this delay, however, was considerably longer with early- than with late-picked fruit, and appeared, therefore, to be associated with the maturity of the fruit at the time of removal from the

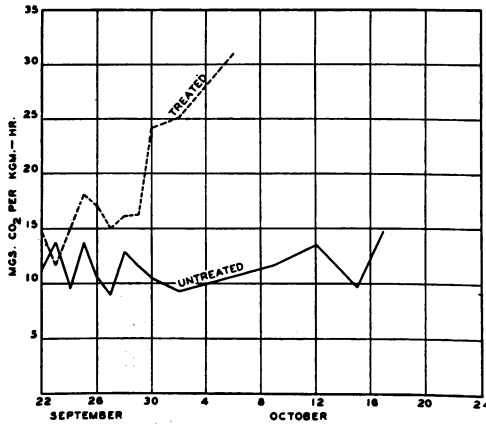


FIG. 2. Effect of gases evolved by ripe pears upon the respiration of Anjou pears treated immediately after picking.

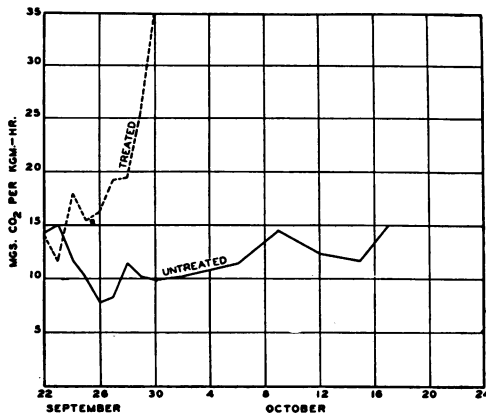


FIG. 3. Effect of gases evolved by ripe pears upon the respiration of Comice pears treated immediately after picking.

tree. Thus in lot III, an early picking of Bartlett, a period of 12 days elapsed in the case of the untreated fruit before an increase in respiration occurred. With fruit gathered at later dates, however, the period between the time of picking and the increase in respiration accompanying ripening became progressively shorter and shorter until with the post-mature lot, the respiratory rate rose rapidly and almost immediately after picking.

It appears, therefore, that fruit in advanced stages of maturity is already at a stage where respiration is tending toward a high rate. Naturally with these pears, little effect from treatments to stimulate respiration can be obtained. Fruit in earlier stages of maturity, however, is either at a stage of low respiratory activity or is tending to drift from a high to a low rate. With these fruits, where the period of low respiratory activity is considerably extended, treatments to stimulate respiration can be expected to be most effective.

As long as the fruit remains attached to the tree, the increase in respiration appears to take place very slowly, and consequently it is considerably beyond the commercial season before the period is reached when pears will no longer be affected by ethylene or naturally occurring gases. Fruit picked during the commercial season was still respiring at a comparatively low rate at the time of picking and showed a marked effect from treatment.

That the effect obtained from ethylene treatment bears a relationship to the maturity of the fruit has also been observed by other workers. WORK (16), for instance, obtained the greatest effect from treatment of tomatoes picked 30 to 40 days from blooming. DAVIS and CHURCH (1) observed that the stimulative effect of ethylene on the respiration of Japanese persimmons declined as the fruit ripened, especially in the last stages and in the stored fruit. With bananas, HARTSHORN (8), using acetylene, found that treatment appeared to be most effective in shortening the initial period of low activity in respiration and consequently the greatest differences were found in those lots in which this period is of greatest duration. None of these workers, however, have associated these differences in responses to ethylene treatment with natural progressive changes in respiration occurring in the fruit during maturation.

KIDD and WEST (9), working with apples, first designated the period of rapid increase in respiration as the "climacteric" and have observed (11) that while the emanations from ripe apples have the property of hastening the onset of the climacteric, such volatile products were ineffective during the post-climacteric period when softening of the flesh and other outwardly indicated ripening processes occurred. With pawpaws, however, WARDLAW and LEONARD (15) have reported that coloring, softening, and final maturation of the fruit are coincident with the climacteric rise. MAGNESS and BALLARD (13) have similarly found that the respiration of Bartlett pears increases rapidly as the fruit ripens. The respiration curves of these writers do not show such an extended low level, however, as has been observed in these experiments. This can probably be explained on the basis that in their experiments a period of 3 to 5 days elapsed between the time of picking and the initial run, and during this delay the fruit had probably been subjected to conditions favorable for ripening. It was observed, however, that early-

picked fruit had a lower initial rate of respiration than late-picked samples, and also that the speed with which respiration rate increased was less rapid in the early-picked than in the late-picked fruit.

COMPARATIVE EVOLUTION OF ETHYLENE AT DIFFERENT PERIODS

In relation to the differences in results obtained with fruit picked at various stages of maturation, it will be interesting to know at what period the greatest production of volatile substances affecting ripening occurs. KIDD and WEST (10) have stated that the evolution of the effective gas is associated with the climacteric in apples and bananas, but later observations (2, 6, 12) have indicated that the pre-climacteric fruit also shows indications of ethylene production. If ethylene is a normal respiratory product in some fruits and plant tissues and shows concomitant increases or decreases with carbon dioxide production, then there are two stages in the life of the pears when evolution of ethylene should be greatest. The first would be in the premature fruit when the rate of respiration is high and the second during ripening when CO_2 production is also maintained at a high level. That there is not a comparable evolution of ethylene at these two periods, however, was indicated by these experiments.

While the presence of ethylene, as indicated by leaf epinasty, could be detected in the premature fruit, the epinastic effect produced by an equal weight of fruit respiring at the same rate, but during the climacteric was strikingly more pronounced. This observation seemed to hold true not only for Bartlett pears, but for the other varieties studied as well. The indications are, therefore, that the evolution of ethylene during the life of the fruit is greatest during the period shortly preceding and including ripening, and is not necessarily concomitant with carbon dioxide production at other periods. Definite proof of this statement, however, must await the development of an accurate method for the determination of small amounts of ethylene.

Since even immature fruit produced a small amount of ethylene as indicated by leaf epinasty, the indications are that no lot of fruit under observation was ever entirely free from the effects of the gas produced within the tissues. In view of this, it appears that the determination of the physiological behavior of fruit entirely isolated from the presence of ethylene will be extremely difficult. The results of these studies indicate, however, that this gas naturally evolved is an important factor to be considered in the respiration and ripening of fruit. The fact alone that there is apparently a very marked increase in the production of ethylene at the beginning of the climacteric would suggest this possibility. Furthermore, the fact that respiration was retarded and ripening was delayed in those lots kept free from an accumulation of any gases by constant aeration, whereas respiration

was increased and ripening hastened in those lots where the gases were allowed to accumulate, strongly indicates that the gas naturally evolved by the fruit, while not necessarily indispensable to ripening processes, does markedly hasten the period when ripening occurs.

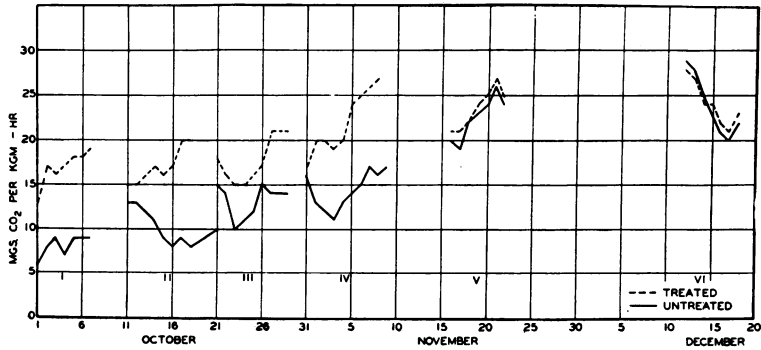


FIG. 4. Effect of ethylene upon the respiration of Anjou pears treated at 65° F. after being held at 37° F. for various periods.

II. HOW LONG AFTER PICKING CAN AN EFFECT FROM ETHYLENE BE OBTAINED?

METHODS.—Since pears are comparatively short lived at high temperatures, it was difficult to determine at just what stage after picking the fruit ceased to respond to ethylene, although the data obtained indicated that treatment was ineffective after the rapid rise in respiration had been nearly completed. In order to follow the changes in response to ethylene treatment after picking more closely, it was decided to use a variety that would show a response over the greatest possible period of time and to further extend this period by the use of cold storage. For this reason, the Anjou pear, a long-lived variety which keeps well under cold storage conditions, was selected. The fruit was picked at commercial maturity from a single tree, and at the time of gathering was in the pre-climacteric stage. Immediately after gathering, two lots were carefully selected for uniformity of size, packed in oil wraps, and stored at 31° and 37° F. respectively. At approximately 12-day intervals, a sample consisting of 30 pears was withdrawn from each lot, taken to the 65° F. ripening room, and treated as indicated in the general methods. As a comparison to the Anjou pear, some studies were also made on Bosc and Comice, which are shorter lived varieties. The method of handling was the same as outlined with the exception that the 37 degree storage lots were not included.

EFFECT UPON RESPIRATION

The period during which the Anjou pears were under observation extended from September 16 to December 12. During this time respiration

data were obtained on six lots removed from storage at consecutive dates. The curves in figure 4, therefore, show, over a fairly long period, the general trends in respiration during ripening of both treated and untreated lots.

That the effect of ethylene upon the rate of respiration steadily decreases after storage is clearly indicated by the results obtained. References to figure 4 show that while there is a very pronounced difference in the rate of

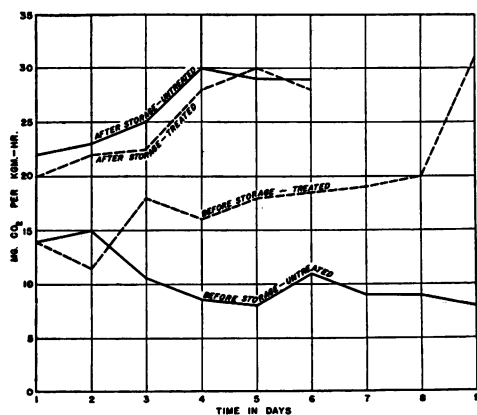


FIG. 5. Effect of ethylene upon the respiration of Comice pears treated before storage and after 6 weeks' storage at 31° F.

respiration between treated and untreated lots after removal from storage early in the season, the magnitude of this difference gradually decreases during a period of two to three months until a point is reached where respiration of the untreated fruit is maintained at as high a level as that of the treated. This period when respiration is no longer affected by ethylene treatment was reached in nine weeks by pears stored at 37° F., while approximately one month longer was required for the pears stored at 31° F. With Comice (fig. 5) no response could be obtained after six weeks, while Bosc were unaffected after only two weeks of cold storage.

Further reference to figure 4 shows that the increase in the rate of respiration, which can be obtained from ethylene over that of untreated lots, is directly correlated with the initial rate or level at which the fruit is respiring at the time of treatment. When this level is low a large increase can be obtained, but when the level is high the difference is correspondingly reduced. Thus, the initial rate of respiration of the first lots removed was at a comparatively low level. The greatest increase was noted in these lots. On subsequent dates, however, there was a gradual increase in the level of respiration of the fruit after removal from storage which was accompanied by a corresponding decrease in effect from ethylene treatment. This trend continued until a point was reached where the fruit, after removal to ripening temperature, was respiring at a high rate, and with these lots no further

increase above the already high rate could be obtained. It appears, therefore, that the rate at which the fruit can carry on respiration is limited to a definite maximum, over and above which no increase can be obtained with ethylene treatment. Furthermore, it appears that there is a gradual drift in respiration toward this high level whether the fruit is held at high temperatures or in cold storage.

EFFECT UPON RATE OF RIPENING

Evidence that the effect of ethylene upon the rate of ripening steadily decreases after storage is shown by the differences in ripening rate between treated and untreated lots removed at the different periods. As shown in table II, of the fruit stored at 37° F. for two weeks, the untreated lot required 17 days to ripen, while the corresponding treated lot ripened in 10 days. After a period of 9 weeks, however, the untreated fruit, upon removal from storage, ripened as rapidly as the treated fruit. Similar results were obtained with the 31° F. series, but with these pears, there was still a slight difference in the number of days required for ripening of treated and untreated fruit after 12 weeks. With Comice, after six weeks' storage, the untreated lots ripened as rapidly as the treated, while Bosc showed no response after two weeks of cold storage.

It might be expected that this observed decrease in the time required for ripening could be due to progress in ripening of the fruit while in storage. On the contrary, the indications were that ripening processes, as outwardly manifested at least, had preceded very little during this period. From all appearances, the fruit which showed no response to ethylene was still nearly as much in an unripened condition as those samples which were markedly affected by ethylene treatment earlier in the season. At the time

TABLE II

EFFECT OF ETHYLENE UPON RESPIRATION AND RIPENING RATE OF ANJOU PEARS AT 65° AFTER REMOVAL FROM STORAGE AT 37° F.

NO. DAYS OF STORAGE	PRESSURE TEST AT TIME OF REMOVAL	INITIAL RATE OF RESPIRATION MG. CO ₂ /KG./HR.		PRESSURE TEST AFTER 9 DAYS' TREATMENT		DAYS TO RIPEN	
		TREATED	UNTREATED	TREATED	UNTREATED	TREATED	UNTREATED
12	19.0	13.63	6.74	3.1	13.9	10	17
24	18.7	15.94	13.53	2.5	8.1	9	15
36	17.8	18.74	14.74	2.5	6.1	8	12
48	17.3	17.09	16.71	2.5	3.7	7	10
60	17.0	20.50	20.00	2.0	2.3	6	6
90	8.5	28.00	29.00	2.0	2.0	5	5

of picking the pressure test of the fruit, an indication of firmness or flesh, was 19 pounds. The pressure test of the first lot failing to respond to treatment was still 17 pounds, indicating that very little softening of the fruit had occurred during storage. The change in the fruit, then, that is correlated with the decrease in effect from ethylene, must be accounted for on another basis.

In table II are shown the trends in initial rates of respiration, the number of days required for ripening, and the pressure tests before and after ripening for both treated and untreated lots of the 37-degree series. As previously pointed out, the initial rate of respiration of the untreated lots, upon removal from cold storage, shows a progressive increase. The number of days required for ripening and the pressure test taken nine days after removal from storage show a steady decline in the untreated lots. The pressure test of the unripened fruit before treatment shows very little decrease for nine weeks, but subsequently a very sudden and marked decline occurs. The striking fact brought out is the correlation existing between the initial rate of respiration and the ripening rate of the fruit. Thus, the period when the fruit has nearly attained its maximum initial rate of respiration upon removal from storage, almost exactly corresponds to the period when the fruit ceases to respond to ethylene treatment. This would indicate that, with these storage pears, the effects of ethylene were confined to the period during which the maximum level in respiration was being attained. Furthermore, indications are that the ripening processes including softening of the flesh, occurring after the peak in respiration had been reached, were not affected by the addition of this gas to the ripening chambers.

That the respiration of pears after delayed periods of cold storage starts off at a markedly higher rate than does that of similar fruit before storage, has been previously shown by MAGNESS and BALLARD (13). These writers also found that an increase in the respiration of Bartlett pears occurred while the fruit was held at 37° F., but no increase was noted with similar fruit held at 31° F. GERHARDT and EZELL (4), however, have found that Comice pears show a consistent increase in respiration during storage at 31° F.

EFFECT OF ETHYLENE UPON PEARS HELD AT COLD STORAGE TEMPERATURES

The results of preliminary investigations, the data of which are not herein included, indicate that ethylene has very little effect upon ripening or respiration of pears held at cold storage temperatures. Bosc, Comice, and Anjou pears treated for periods of from one to three months at 31° and 37° F. showed no apparent increase in respiration or rate of ripening over that of the untreated lots. The indications are, therefore, that the slow progress of the climacteric at low temperatures is not influenced by an increase in the concentration of ethylene over that naturally produced within the fruit tissue.

These results are in agreement with those of KIDD and WEST (12) who have observed that the respiration of apples was not increased at cold temperatures upon exposure to the vapors produced by ripe fruit. DUSTMAN (3), however, has reported increased rate of ripening of Ben Davis, Rome Beauty, and Stayman apples treated with ethylene for one month at cold storage temperatures.

Discussion

The time of ripening in relation to the occurrence of the climacteric is obviously different with pears held under cold storage conditions than with fruit held at higher temperatures. It will be recalled that with newly-picked pears, ripening symptoms were first apparent with the start of the climacteric; and before the peak of respiration had been reached, the fruit had become fully ripe. With storage fruit, however, while apparently the climacteric occurred at cold temperatures, ripening was delayed until the post-climacteric period or until the fruit was removed to temperatures favorable for ripening. Since the factors associated with the climacteric rise are not clearly understood at the present time, an explanation for the differences observed is not readily apparent. It is clearly indicated, however, that fruit picked in a stage of low respiratory activity tends to drift toward a high maximum rate whether held at high or low temperatures, and while the increase in respiratory activity may occur independently of the ripening of the fruit, activation of the ripening processes appear to be closely associated, at least, with the incidence of this period.

The results obtained with both pre- and post-storage pears indicate that the effects of ethylene are definitely confined to a period preceding the stage in the life of the fruit when the maximum level in respiration has been reached. With fruit treated immediately after picking, the greatest response from treatment was obtained with those fruits having the greatest length of time elapsing before the climacteric, while the least response was obtained in those cases when at the time of picking this rise in respiration had started or was well under way. The results obtained with fruit treated after delayed periods of storage clearly show that ethylene treatment is effective only during the period of ascending respiratory activity, and the magnitude of the response obtained bears a direct relationship to the increase in respiration that has naturally taken place prior to treatment.

The marked differences observed in the periods of time during which long- and short-lived varieties of pears respond to ethylene treatment can be cited as further evidence that ethylene treatment is effective during only a definite period in the life of the fruit. With the Anjou pears, a long-lived variety, over 12 weeks of storage at 31° F. had elapsed before the fruit ceased to respond to treatment. With the Bosc pear, a short-lived variety,

no effect from ethylene could be obtained after two weeks of storage. An intermediate-lived variety, the Comice, showed no response after six weeks of storage. Furthermore, Anjou pears stored at 31° F. responded to ethylene treatment over a period of four weeks longer than did pears of the same variety held at 37° F. It appears, therefore, that with all the varieties of pears studied, there is a gradual drift toward a period of a maximum level in respiration, and when this stage in the life of the fruit has been reached, ethylene treatment is no longer effective. While external factors such as changes in temperature do retard or accelerate the rate at which this increase in respiration takes place, the general trend followed appears to be controlled primarily by internal factors inherent within the fruit.

Since a trend in respiration during maturation similar to that shown by pears has been observed to occur in a number of other fruits (5, 9, 14, 16), it may possibly follow that these fruits would similarly respond to ethylene treatment only during a definite life period. This being true, the wide diversity of results that have been obtained from the use of ethylene as a ripening agent can be at least partly explained on this basis.

Summary

1. The emanations from ripe pears have been found to increase the rate of respiration and ripening of newly-picked Bartlett, Comice, and Anjou pears. The greatest effect from treatment was obtained with fruit picked early in the season, and the least effect with fruit picked at post-mature stages.

2. The production of ethylene, as indicated by leaf epinasty, appears to increase during the period of ascending respiratory activity accompanying ripening of the fruit.

3. No increase in respiration or ripening could be obtained from ethylene treatment of fruit which had been held in cold storage for certain periods. Long-lived varieties responded over a greater period than did short-lived varieties. Pears held at 31° F. responded for a greater length of time than did similar fruit held at 37° F.

4. The decrease in effect from ethylene treatment appears to be associated with a natural increase in the respiratory activity of the fruit after picking.

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