# ROLE OF ETHYLENE IN THE INITIATION OF FRUIT RIPENING

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## SUMMARY

The green-life of bananas has been shown to be reduced by short periods of exposure to ethylene, the length of exposure and ethylene concentration being such that ripening was not immediately initiated, but commenced several days or even weeks after fruit had been treated. This effect is proposed as evidence that endogenous ethylene is physiologically active throughout the preclimacteric life of a fruit, and that it is functional in determining when the climacteric will occur.

The sensitivity to ethylene, defined as the proportional loss in green-life brought about by an exposure to the gas, is shown to vary exponentially with green-life at harvest, decreasing as fruit approach maturity. The sensitivity of harvested fruit is shown to increase with time after harvest, the rate of increase appearing to be dependent on the green-life at harvest.

The concept that endogenous ethylene initiates ripening by exceeding a critical threshold level is discussed and proposed to be inadequate.

## I. INTRODUCTION

Two concepts regarding the function of ethylene have been proposed. Kidd and West (1933, 1945) and Hansen (1943) proposed that it functions as a ripening hormone, Kidd and West (1945) suggesting that a minimum threshold concentration in the tissues is necessary before respiratory activity is affected. It has also been proposed that it is simply a by-product of the ripening process (Biale, Young and Olmstead 1954; Biale 1960a, 1960b). Recent evidence (Burg and Burg 1962a, 1962b, 1965b, 1966; Hansen 1966; Mapson and Robinson 1966; Pratt and Goeschl 1968) has added much weight to the theory that it acts as a ripening hormone.

In attempts to resolve which concept is correct a great deal of work has been done to determine whether physiologically active concentrations of ethylene occur within fruit prior to the onset of the climacteric rise. A concentration was accepted as being physiologically active if it could be shown to hasten respiration and ripening (Burg and Burg 1962a). It appears that this approach has caused many investigators (Kidd and West 1945; Burg and Burg 1962a, 1962b, 1965a, 1965b;

Lyons, McGlasson and Pratt 1962; Pratt and Goeschl 1969) to accept the concept that a critical threshold level of ethylene must occur within a fruit before ripening will be initiated. It is suggested by Burg and Burg (1962a) that a concept of a critical triggering concentration of ethylene being required to produce a response has been inferred from the observation that ethylene is without effect on most fruits if it is applied after ripening has commenced.

This paper presents data obtained with bananas which provide additional evidence that ethylene functions as an endogenous ripening hormone. It also discusses the concept of a critical threshold level being necessary for the initiation of fruit ripening and suggests that the concept is inadequate.


## II. MATERIALS AND METHODS

The bananas used in this investigation (*Musa acuminata* Colla cv. Giant Cavendish, Queensland synonym Mons Mari: Simmonds 1959) were obtained locally on the day of harvest.

Ethylene concentrations were obtained by mixing ethylene and air in the desired proportions using manometric capillary flowmeters. When very low ethylene concentrations were required, the dilution was carried out in two stages, ethylene mixtures being made up in pressure cylinders by compression with nitrogen and then diluted as above. All ethylene concentrations were finally monitored using an Aerograph 204 Gas Chromatograph with flame ionisation detector and aluminium oxide column.

Green-lives (see below) of fruit were either determined by monitoring respiration rates using an infrared gas analyser to detect the onset of the climacteric rise (experiment 1) or estimated by observing when the first detectable change in skin colour occurred (experiments 2, 3, 4). The latter can be used, since it has been shown (Peacock 1966) that the time interval between the onset of the climacteric and the commencement of colouring is constant, provided temperature is kept constant, over a wide range of green-lives. In the data presented, no correction factor has been used to allow for this time interval. All experiments were conducted at 20°C.

*Experiment* 1.—Twenty-four bananas were held in individual containers and ventilated with humidified air using manometric capillary flowmeters. Twelve of these fruit were exposed to 0·44 p.p.m. of ethylene for the first 4 days after harvest.

*Experiment* 2.—Fruit were exposed to 0·32 p.p.m. of ethylene for 0, 1, 2, 3, 4, 5 and 6 days and then held in air until ripening commenced. During this stage fruit were held in a specially designed cabinet, not in individual containers, in which they are ventilated with humidified, temperature controlled, ethylene-free air at a rate in excess of 100 ml/min/fruit. Four hands from the same bunch were used and divided so that three fruit from each hand were in each treatment. The experiment was replicated using another bunch.

*Experiment* 3.—Fruit were selected from each of 10 bunches of bananas, the bunches judged to be at different stages of maturation on the basis of fullness of fingers. Ten fruit from each bunch were held in air (as in experiment 2), while another 10 were treated with 0·32 p.p.m. of ethylene for 4 days before also being held in air.

*Experiment* 4.—Twenty-one fruit were chosen from each of two hands of a bunch. Six fruit were held continuously in air as in experiment 2, while the remainder, six fruit per treatment, were exposed to 0·32 p.p.m. of ethylene for 2 days after first being held in air for 0, 1, 2, 3, 4, and 5 days. After ethylene treatment, fruit were returned to air. The experiment was replicated using another bunch.

*Definitions.—*

*Green-life* was defined by Peacock and Blake (1970) as the time a harvested fruit takes to reach maturity under defined conditions. The same meaning is assigned to the term throughout this paper.

*Maturity,* as defined by the above authors, is the stage of biochemical development which a fruit has reached when the climacteric rise commences.

*Sensitivity to ethylene* is defined as the proportion of green-life lost due to an exposure to ethylene. In the data presented here, green-life lost has been expressed as a proportion of initial green-life.

## III. RESULTS

The data from experiment 1 are shown in Table 1. It can be seen that this short exposure to ethylene, though insufficient to initiate ripening, has shortened green-life by 32%, a highly significant ($P < 0.001\%$) amount.

### TABLE 1

EFFECT OF A SHORT EXPOSURE TO ETHYLENE (0·44 P.P.M. FOR 4 DAYS) ON THE GREEN-LIFE OF BANANAS (EXPT. 1)

Green-life in Days

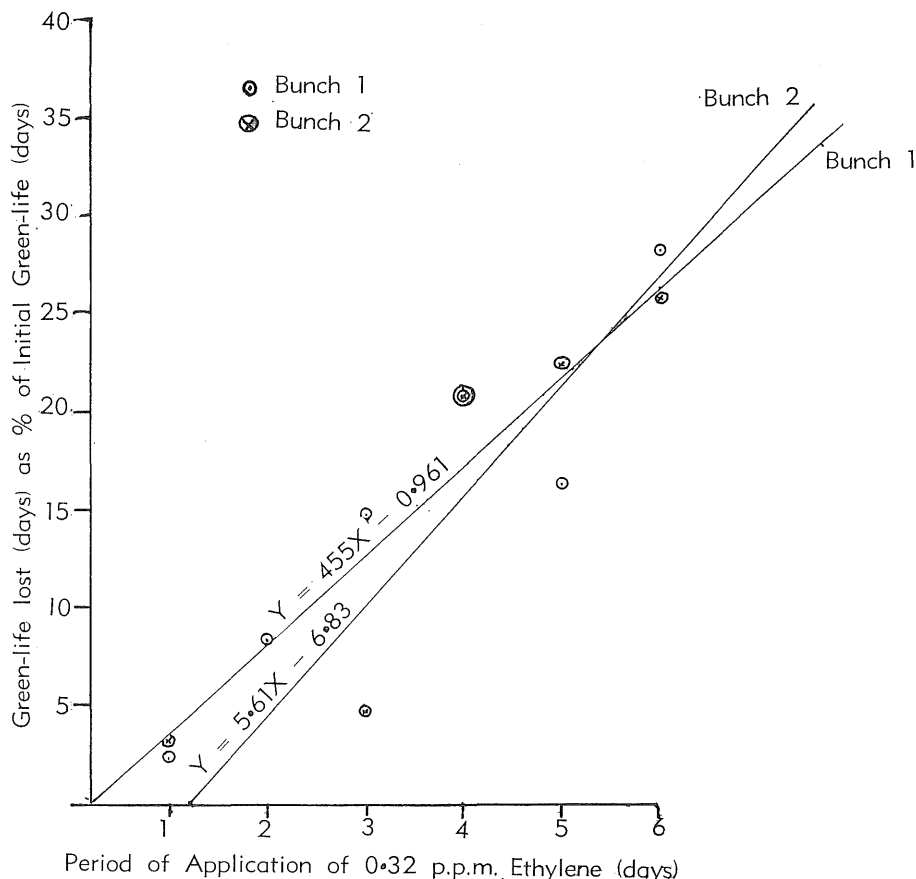| Control | Ethylene Treated |
|---|---|
| 36·5 | 24·2 |
| 37·5 | 24·3 |
| 36·6 | 21·2 |
| 49·0 | 22·3 |
| 38·2 | 24·3 |
| 47·1 | 25·2 |
| 29·4 | 24·1 |
| 43·3 | 36·3 |
| 40·9 | 25·3 |
| 37·5 | 26·2 |
| 39·3 | 25·3 |
| 31·5 | 36·2 |
| Mean  ..  38·9 | 26·4 |
| S.E.  =  1·566 | S.E.  =  1·345 |

Fig. 1.—Effect of period of application of ethylene on loss of green-life. (Note that $4 \cdot 55X$ is shown incorrectly as 455X.)

The results of experiment 2 are shown in Figure 1. Again, the short exposures to ethylene have reduced green-life to a highly significant ($P < 0 \cdot 001\%$) extent, thus confirming the result of experiment 1. The reduction in green-life that occurs is related to the period for which the ethylene is applied, apparently in a linear manner.

Application of $0 \cdot 32$ p.p.m. of ethylene for 4 days (experiment 3), to fruit having different green-lives at harvest, results in fruit being exposed to ethylene for different proportions of their preclimacteric life. The data would more clearly show the effect of ethylene if fruit of different green-lives were exposed for a constant proportion of their lives. Since the reduction in green-life is proportional to the time of application (experiment 2) the data (Figure 2) have been corrected by simple proportion to show the green-life that would be lost if fruit were exposed to $0 \cdot 32$ p.p.m. of ethylene for $12 \cdot 7\%$ of their lives, being the equivalent of a 4-day exposure to a $31 \cdot 5$ day fruit. It can be seen (Figure 2) that the short exposure has significantly reduced green-life, thus again confirming the data of the first two experiments. The degree of shortening, i.e. the sensitivity of the fruit to ethylene, increases with increasing green-life (significant at the 1% level) apparently in an exponential manner.
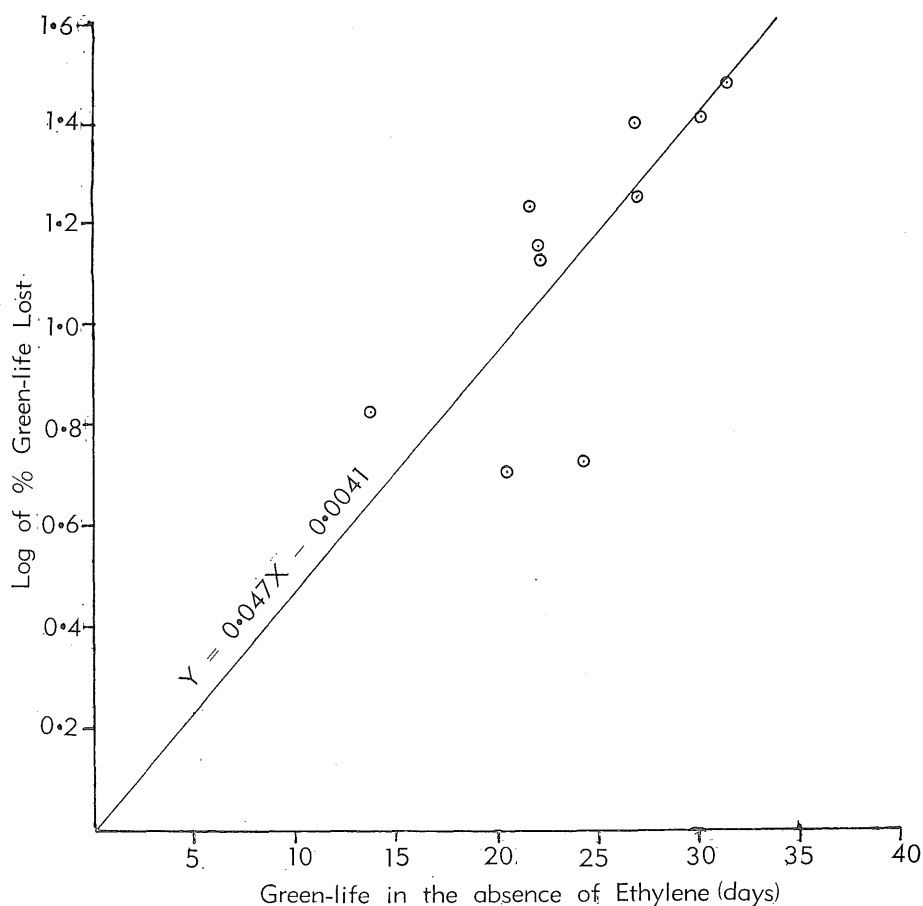
Fig. 2.—Relationship between sensitivity to ethylene and green-life at harvest.

When the application of ethylene is delayed for some time after harvest (experiment 4—Figure 3), the reduction in green-life that occurs is found to increase (significant at $P < 0.001\%$). This thus indicates that as harvested fruit approach maturity their sensitivity to ethylene increases (cf result experiment 3). The increase appears to be linearly related to time but this may be only due to the narrow time period examined. A comparison of the data obtained from each bunch shows that the rate at which sensitivity changes appears to be a function of the green-life of the bunch, there being a significant difference ($P < 0.01\%$) in the rates for the two bunches.

Using the time to the onset of colouring as a measure of green-life, as has been done in experiments 2, 3 and 4, introduces an error in the data as presented. To obtain actual green-life figures, a constant would have to be subtracted from all data (Peacock 1966). If this were done, the data would be altered differentially, that for the more mature fruit being altered most. In experiment 2 this would only increase the level of significance of the data. In experiment 3,
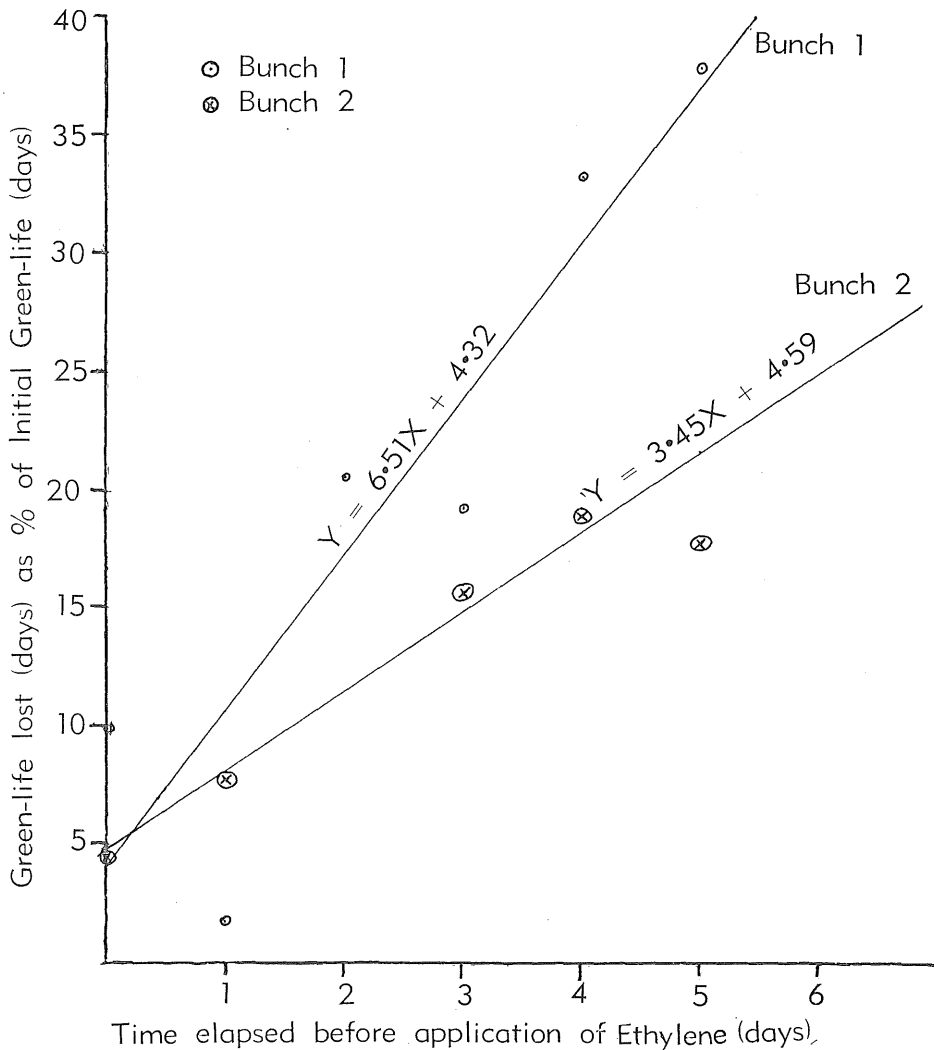
Fig. 3.—Effect of a 2-day exposure to 0.32 p.p.m. of ethylene applied at different times after harvest.

however, the level of significance of the data would be reduced. To check whether or not this reduction would be substantial, a constant of $3 \cdot 0$ days was assumed—values for this constant found by Peacock (1966) ranged from $2 \cdot 4$ to $3 \cdot 1$—and applied to the data of this experiment as a correction factor. The data still showed a significant $(P < 0 \cdot 05)$ increase in the sensitivity of fruit with increasing green-life.

When such a constant correction factor is applied to the data of experiment 4, the rate of change of sensitivity is increased, becoming more significant. The difference between the rates of change of sensitivity for the two bunches is reduced, but not to a significant extent due to their long green-lives (15 and $28 \cdot 7$ days).

## IV. DISCUSSION

Burg and Burg (1962*a*) have shown that the addition of ethylene to mangoes after the climacteric commenced hastens ripening, and hence it can be said that ethylene is physiologically active during this stage of ontogeny, at least on some of the processes that occur. That similar behaviour could not be demonstrated in all fruits was ascribed by these authors to the fact that in some fruits the rate of ethylene production increases abruptly coincident with the onset of the climacteric and they quickly acquire optimal internal levels by their own metabolism. However, the problem that has caused much discussion over recent years is whether ethylene is active in the initiation of these processes. As Pratt and Goeschl (1969) have stated, "Students of the role of ethylene in fruit ripening have tended to confuse two separate phenomena related to ethylene—ethylene as the trigger of ripening and the excess production of ethylene which accompanies ripening, the climacteric and final senescence."

As stated earlier, in attempting to answer this question many investigations have been aimed at determining whether the ethylene levels within fruit prior to the onset of the climacteric rise are physiologically active, a concentration being accepted as physiologically active if it could be shown to hasten respiration and ripening (Burg and Burg 1962*a*). As these authors point out, "because the response of a fruit to ethylene is related to the log of the gas concentration, and since the internal concentration of ethylene will be augmented by approximately the concentration of gas maintained in the ambient atmosphere, it follows that a response cannot be demonstrated easily unless the applied concentration equals or exceeds that within the fruit". Hence, just because an applied concentration cannot be shown to have an effect, it does not mean that no effect exists.

If ethylene were only physiologically active at or just prior to the onset of the climacteric, then treating fruit with short exposures to ethylene well before this stage should have no effect. That this is not the case has been clearly demonstrated (Table 1, Figures 1, 2 and 3). This result suggests that endogenous ethylene throughout the life of a fruit is functional in determining when the climacteric is initiated and is quite rightly regarded as a plant hormone. This possibility was recognized by Burg and Burg (1962*b*). A similar result has been obtained by Vendrell (1970*b*) who, quoting his own unpublished data, stated that "bananas subjected to a temporary ethylene treatment ripen sooner than controls".

Further evidence that endogenous ethylene is physiologically active well prior to the onset of the climacteric could perhaps be obtained by holding fruit under reduced pressure or in the presence of a helium/oxygen mixture for short periods (Burg and Burg 1965*a*, 1966). Such treatments should not be maintained until ripening commences, otherwise the results could be interpreted as being due to either reduced endogenous ethylene levels throughout the storage life of the fruit or just prior to the onset of the climacteric rise.

From the data of Figure 1 it can be seen that the green-life lost by a fruit due to an exposure to ethylene was a function of the time of application. Several investigators (Biale 1960*a*; Burg and Burg 1962*a*) have shown that the time fruit take to reach the climacteric peak is logarithmically related to the concentration of applied ethylene. In both investigations, ethylene was applied continuously. It appears therefore that the effectiveness of ethylene in reducing green-life of a fruit is a function of both the period of application and log concentration. If this is so, then what is meant by a minimum threshhold level of ethylene would appear to be that concentration which initiates ripening in such a short time that the interval is non-detectable by present experimental techniques; being just a function of experimental accuracy, it has no physiological significance.

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It appears therefore that ethylene is functional in two roles, one in which it determines the green-life of a fruit and the other where it affects the rate of various ripening processes once they have commenced. A similar conclusion was reached by Frenkel, Klein and Dilley (1968), who suggested that one role may be as an effector of specific protein synthesis and another as a product of ripening metabolism involving some of the same proteins.

Burg and Burg (1965b) examined the effects of continuous application of low levels of ethylene to bananas. They interpreted their results to mean either that the production of ethylene by the fruit increases as it nears the onset of the climacteric, and that the addition of ethylene raises the internal concentration above the necessary minimum threshold level, or that the sensitivity of the fruit to ethylene changes, increasing as the fruit approaches the onset of the climacteric. These authors do not define the term "sensitivity", but from their data they apparently mean the ratio of the amount of green-life lost while under ethylene to the amount of green-life that was remaining at the time the ethylene was applied.

It has been shown (Figure 3) that the sensitivity (as earlier defined) of harvested fruit does increase as their remaining green-life decreases; hence Burg and Burg's (1965b) interpretation of their data is correct. It has, however, been shown that the sensitivity of a fruit to ethylene (Figure 2) increases as its green-life increases, which appears to be a direct contradiction to the data of Figure 3. It appears that as fruit approach maturity while attached to the tree—the data of Figure 2 can be taken as a measure of this—their sensitivity decreases, but that it increases as the fruit approach maturity off the tree (Figure 3). This paradox can be explained by examining the data of Figure 3. Two bunches of quite different stages of maturation have been used (green-lives of 15 and 28·7 days). As these data show, the rate of change of sensitivity with time of the more mature bunch is significantly less than that of the immature one. Such a situation makes the result obtained in experiment 3 (Figure 2) inevitable. It should be noted that these sensitivity changes may only reflect, at least in some cases, changes in the natural endogenous ethylene level. Pratt and Goeschl (1968) produced data with honey-dew melons which suggest that this is the cause of apparent sensitivity changes in that fruit. To be a suitable explanation for the data presented here, however, it would mean that changes in the endogenous ethylene level of bananas would have to commence from the time of harvest.

Gazit and Blumenfeld (1970), working with avocadoes, have shown that this fruit is relatively insensitive to ethylene immediately after harvest, in that a short exposure (48 hr) to a high ethylene concentration (50 p.p.m.), much higher than is naturally present in preclimacteric fruit, does not initiate ripening immediately. A similar result has been found in this laboratory with bananas. Fruit exposed to 60 p.p.m. of ethylene for 6 hr did not commence ripening nor was any reduction in their final green-lives detected. Gazit and Blumenfeld (1970) have also demonstrated that the sensitivity of this fruit to ethylene also increases rapidly after harvest. These results support the contention of Burg and Burg (1964) that while a fruit is attached to a tree it receives a ripening inhibitor from the tree which inhibits its response to ethylene.

Recent evidence (Vendrell 1969, 1970a, 1970b) indicates that the inhibitor is probably indole acetic acid (IAA) or possibly some other natural hormone. This is further suggested by the data of Valdovinos, Ernest and Henry (1967), which indicate that ethylene can function as an inhibitor of IAA synthesis and hence endogenous or applied ethylene possibly acts through modifying the endogenous IAA concentration.

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