INHIBITION OF ETHYLENE PRODUCTION IN BANANA FRUIT TISSUE BY ETHYLENE TREATMENT

By M. VENDRELL*† and W. B. MCGLASSON*

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Abstract

A temporary ethylene treatment, sufficient to stimulate ripening in banana fruit tissue, partly suppresses endogenous ethylene production and the evolution of ethylene from methionine. The production of endogenous ethylene does not return to rates normal for naturally ripening fruit after the exogenous ethylene is removed. The extent of inhibition is related to the concentration of applied ethylene up to 5–10 p.p.m., and to the duration of treatment within the period 12 hr to 3 days. Other characteristics of ripening appear to develop normally, except in the shorter treatments, where respiration shows a lower climacteric peak and chlorophyll breakdown is delayed.

The responses of separated peel and pulp sections to exogenous ethylene were also examined. Pulp sections ripened and showed suppression of endogenous ethylene as did whole slices. Peel sections showed a climacteric-like respiratory response to exogenous ethylene but ethylene production remained at the levels of non-gassed sections following either a 1-day or a 3-day treatment. It is suggested that ripening of the peel in whole fruit depends on the ethylene produced by the pulp.

Exogenous ethylene also reduces the amount of endogenous ethylene induced by infiltration with 2,4-dichlorophenoxyacetic acid. In this instance the effect is dissociated from ripening, which is delayed by the auxin, and suggests that the auxin-induced ethylene production arises via the same pathways involved in natural ripening or ones equally susceptible to suppression by exogenous ethylene.

I. INTRODUCTION

The onset of natural ripening in bananas is indicated by a sharp increase in ethylene production followed within a few hours by the climacteric rise in respiration. Peak ethylene production is reached shortly before the climacteric peak (Burg and Burg 1965*a*). Conversion of starch to sugar, softening, and disappearance of chlorophyll begin during the climacteric rise and are largely completed within a few days after the climacteric peak. In fruits of the climacteric type such as the banana, ethylene is considered to be the endogenous hormone responsible for the initiation of ripening (Pratt and Goeschl 1969). Once ripening is underway in these fruits amounts of ethylene in excess of those considered necessary for the initiation of ripening are evolved (Pratt and Goeschl 1969). Recently, however, evidence has been presented showing the existence of ethylene-dependent processes in banana fruit after ripening has been initiated (Hesselman and Freebairn 1969).

* Plant Physiology Unit, Division of Food Research, CSIRO, and School of Biological Sciences, Macquarie University, North Ryde, N.S.W. 2113.

† Present address: Departamento de Química Macromolecular, E.T.S. Ingenieros Industriales, Barcelona-14, Spain.

Hitherto it has been accepted that the application of exogenous ethylene to fruits of the climacteric-type induces ripening biochemically indistinguishable from that which occurs under the influence of endogenous ethylene (Pratt and Goeschl 1969), and also that such an application stimulates the synthesis of endogenous ethylene (Burg and Burg 1965*b*). This paper shows that banana fruit may be an exception to these generalizations. Data are presented which indicate that application of exogenous ethylene may partially suppress endogenous ethylene production.

II. Material and Methods

(*a*) *Preparation and Treatment of Whole Bananas and Slices*

Mature green bananas of the Williams cultivar of the Intermediate Cavendish group were obtained from Avoca, N.S.W. The handling of whole bananas and the preparation of transverse slices (6 mm thick) have been described by Palmer and McGlasson (1969). Sections of pulp tissue were prepared by removing the peel from 6-mm slices. Sections of peel tissue about 3 cm square were cut from whole bananas and freed of adhering pulp tissue. Composite samples (20–25 g) of tissue were used. Banana slices were treated with aqueous solutions of 2,4-dichlorophenoxyacetic acid (2,4-D) and [^{14}C]methionine by vacuum infiltration (66 cmHg for 1 min). Ethylene treatment has been described by Vendrell (1969). All measurements were made at 20°C.

(*b*) *Analytical Procedures*

Carbon dioxide production was measured colorimetrically (Claypool and Keefer 1942) or with an infrared gas analyser, model SB2 (Grubb Parsons & Co. Ltd., England). Ethylene was assayed by gas chromatography (McGlasson 1969). Endogenous ethylene production by samples following treatment with ethylene was measured after flushing out the exogenous ethylene. This was accomplished by ventilating the respiration jars with air streams free of ethylene or by applying a vacuum (66 cmHg for 1 min) to the tissue. The latter treatment has no adverse effects on unripe banana tissue. Soluble solids were measured as described by Palmer and McGlasson (1969). A measurement of degreening of the skin (chlorophyll breakdown) was made using the colour index devised by the Fruit Dispatch Co. (Anon. 1961).

(*c*) *Radiotracer Methods*

[1,2,3,4-^{14}C]L-Methionine was obtained from the Radiochemical Centre, Amersham, England. $^{14}CO_2$ evolved by slices infiltrated with labelled methionine was measured after collecting the respired CO_2 in 9 ml ethanolamine–methoxyethanol (1 : 4 v/v) (Palmer and McGlasson 1969). $^{14}C_2H_4$ was measured after collecting the evolved ethylene in 9 ml of 0·25M mercuric perchlorate in 2M perchloric acid at 0°C. 1 ml of this trapping solution was mixed with 9 ml of scintillator solution which consisted of a mixture (2 : 1 v/v) of toluene scintillator [4 g 2,5-diphenyloxazole and 0·1 g 1,4-bis(4-methyl-5-phenyloxazol-2-yl)benzene in 1 litre of toluene] and Triton X-100*. The samples were counted in a liquid scintillation spectrometer (Tricarb model No. 3325, Packard Instrument Co., Illinois, U.S.A.). Counting efficiency was 69%.

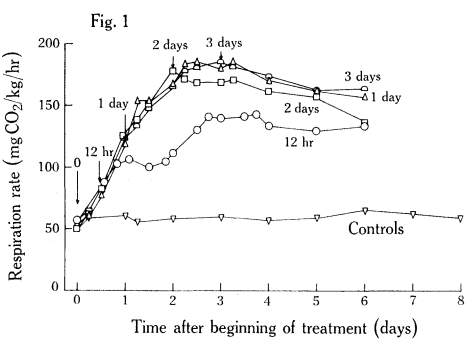
III. Results

(*a*) *Effect of Different Periods of Treatment with 10 p.p.m. Ethylene on Respiration, Ethylene Production, and Ripening in Whole Fruit and Slices*

Treatment for 12–15 hr with 10 p.p.m. ethylene is usually required to induce ripening in slices (Vendrell 1969). Shorter treatments may cause temporary increases in respiration which return to rates normal for green fruit slices after about 1 day.

* Supplied by Rohm and Haas Co., Pennsylvania, U.S.A.

Figure 1 shows the respiratory responses of slices treated with 10 p.p.m. ethylene for intervals of up to 3 days. Short treatments (less than 12 hr) which may cause a partial stimulation of respiration but do not induce ripening (Vendrell 1969) have no effect on subsequent endogenous ethylene production. In treatments that induced ripening, endogenous ethylene production followed a different pattern and was lower than that measured during natural ripening (Fig. 2). Immediately after a 12-hr treatment ethylene production was very low. It then rose to a peak 6 hr after the end of the ethylene treatment, followed by a decline to low levels. A further rise began between 19 and 24 hr and a second larger peak occurred about 36 hr after the end of the ethylene treatment. In other experiments a partial vacuum was applied to remove exogenous ethylene remaining in the tissue. This treatment decreased the first peak slightly, but it did not eliminate it. A distinct peak was observed even after the vacuum treatment was repeated two or three times.



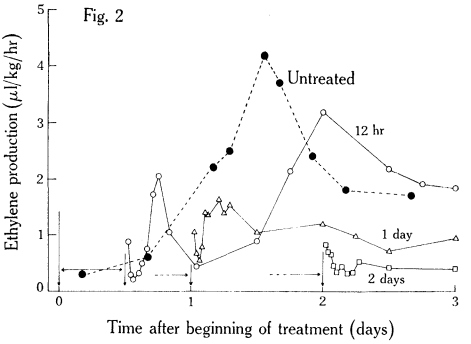


Fig. 1.—Respiration rates of slices treated with 10 p.p.m. ethylene for different periods. Each curve represents the average of three composite samples, each of four 6-mm slices, cut from four matched fruit. The vertical arrows indicate the duration of the ethylene treatments.

Fig. 2.—Ethylene production by slices after treatment with ethylene (10 p.p.m.) for different periods. Exogenous ethylene was removed by transferring the jars to ventilating air streams free of ethylene. The data apply to the same samples referred to in Figure 1. The broken line was superimposed to show the magnitude and time course of ethylene production by naturally ripened (untreated) slices. These slices began to ripen about 11 days after cutting. Other details as for Figure 1. S.E. of mean ($n' = 3$): 12-hr treatment, $\pm 0{\cdot}17$ (36 d.f.); 24-hr treatment, $\pm 0{\cdot}28$ (30 d.f.); 48-hr treatment, $\pm 0{\cdot}074$ (24 d.f.); untreated slices during ripening, $\pm 0{\cdot}29$ (18 d.f.). Mean of steady values for untreated slices before ripening $0{\cdot}076$ μl/kg/hr ($n' = 27$), S.E. of mean $\pm 0{\cdot}020$ (18 d.f.).

Slices treated for 1 day showed an initial peak in ethylene production at about 5 hr but did not develop a distinct second peak; a 2-day treatment stimulated ethylene production but the rates were lower than those for slices treated for 1 day. These lower levels of ethylene production continued during the first 4–5 days of the respiratory climacteric.

The non-gassed slices of the same batch used in Figures 1 and 2 began to ripen 11 days after cutting. In another experiment the control slices took 24 days to begin to ripen and a 12-hr treatment with ethylene was not sufficient to induce ripening. A 24-hr treatment gave about the same response in terms of respiration and ethylene production as a 12-hr treatment in the previous experiment.

The responses of whole bananas were similar to those of slices but less pronounced, probably because of less efficient removal of exogenous ethylene from the tissue. A 12-hr treatment also gave two peaks in ethylene production.

The pulp of all samples induced to ripen by ethylene treatment (at least 12 hr) ripened in the same manner. Four and 6 days after the climacteric commenced total soluble solids were about 18 and 22% respectively. Softening of the pulp and the presence of aroma were similar in samples treated and not treated with ethylene. However, differences were observed in the softening and yellowing of the skin. In bananas treated for 15 hr (Fig. 3) there was a significant delay in reaching N5 of the

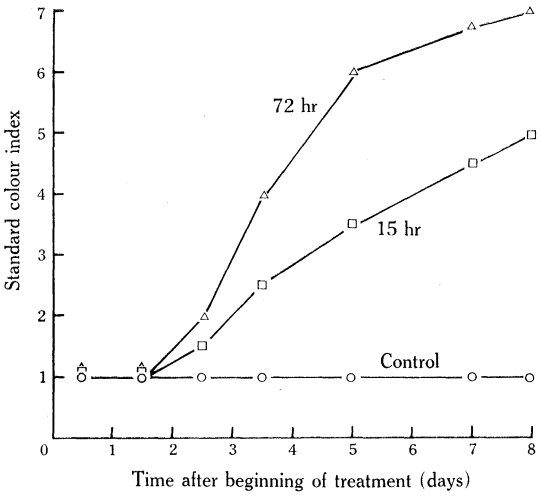


Fig. 3.—Yellowing of the skin of whole bananas treated with ethylene (10 p.p.m.) for different periods. (Standard colour index, Anon. 1961.) Each curve represents the average of three bananas.

Standard Colour Index, compared with bananas treated for 3 days, which behaved like fruit ripened naturally. Although smaller, there was also some delay in fruit induced to ripen with a 1- and 2-day ethylene treatment.

(*b*) *Effect of Treatment with Different Ethylene Concentrations for Varying Periods on Respiration and Ethylene Production by Slices*

Banana slices were treated with 1, 5, and 30 p.p.m. ethylene for 1 and 3 days (Fig. 4). A 3-day treatment with the three concentrations resulted in the development of the normal climacteric pattern of respiration. A 1-day treatment with 1 p.p.m. resulted in a lower maximum. With 5 and 30 p.p.m. the rates of respiration were the same compared with a 3-day treatment, except for a slight change when the ethylene treatment stopped.

The rates of endogenous ethylene production subsequent to these treatments depended on exogenous ethylene concentration and the duration of treatment (Fig. 5). Slices treated with 1 p.p.m. for 1 day showed the same pattern of ethylene production observed for slices treated with 10 p.p.m. for 12 hr (Fig. 2). Treatment with 5 and 30 p.p.m. for 1 day suppressed endogenous ethylene production more than 1 p.p.m. for 1 day, but 30 p.p.m. were not more effective than 5 p.p.m. After a 3-day treatment with the three concentrations, ethylene production was smaller than for

the corresponding 1-day treatments. In another experiment using ethylene concentrations of 1, 3, 10, and 30 p.p.m., ethylene production 6 hr after a 3-day treatment was 1·76, 0·78, 0·35, and 0·27 μl/kg/hr respectively. Thus the concentrations above 5 p.p.m. cause little further suppression of endogenous ethylene production.

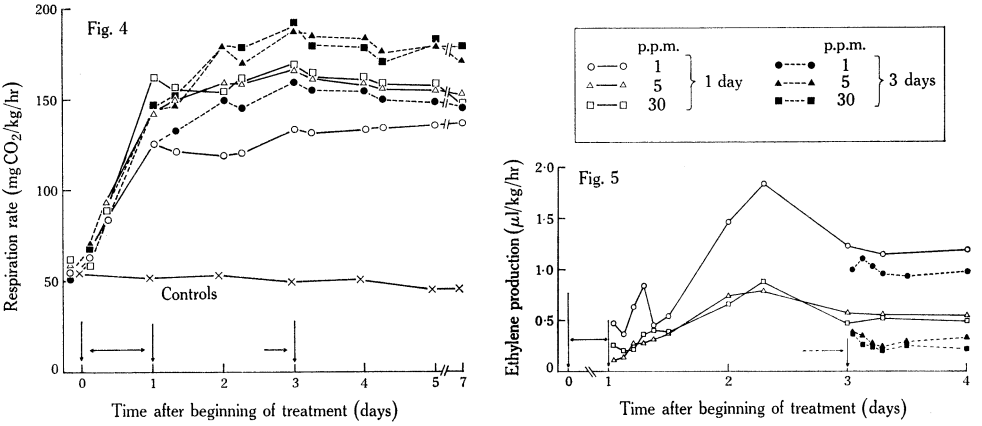


Fig. 4.—Respiration rates of banana slices treated with different ethylene concentrations for different periods. Other details as for Figure 1. See box for explanation of symbols.

Fig. 5.—Ethylene production by banana slices after treatment with ethylene at different concentration and for different periods. Vacuum (66 cmHg for 1 min) was applied to remove exogenous ethylene. The data were obtained from the same samples used in Figure 4. Other details as for Figure 1. S.E. of mean ($n' = 3$): 1 p.p.m. for 1 day, ±0·061 (24 d.f.); 5 p.p.m. for 1 day, ±0·041 (21 d.f.); 30 p.p.m. for 1 day, ±0·050 (19 d.f.); 1 p.p.m. for 3 days, ±0·11 (14 d.f.); 5 p.p.m. for 3 days, ±0·042 (14 d.f.); 30 p.p.m. for 3 days, ±0·0069 (14 d.f.). Mean of steady values for untreated slices before ripening 0·020 μl/kg/hr ($n' = 30$), S.E. of mean ±0·020 (20 d.f.). The pattern and values of ethylene production by untreated slices during ripening were similar to those shown in Figure 2. See box for explanation of symbols.

(c) *Effect of Cutting on Respiration, Ethylene Production, and Ripening of Pulp and Peel Tissue*

Both sections of peel and pulp showed after cutting a peak of induced respiration (Fig. 6), as observed with whole slices (Palmer and McGlasson 1969) but the peak was higher in pulp sections. The respiration rates of peel and pulp sections subsequently decreased and stabilized at low levels. The ratio of the area of cut surface per unit fresh weight of tissue was about 3·3 cm^2/g for peel sections and 4·3 cm^2/g for pulp sections.

Ethylene production by sections of peel and pulp also passed through a peak (Fig. 7) as observed in slices (McGlasson 1969), but the size of the peak for the pulp tissue was about three times that of the peel and slightly delayed. The rates of ethylene production stabilized at lower levels in the peel than in the pulp.

The sections of pulp developed a respiratory climacteric (Fig. 6). Ripening also took place as indicated by softening, presence of aroma, and sugar production. The time taken to reach the respiratory climacteric was about the same in pulp tissue as

in whole slices, but the rates of respiration per unit fresh weight during the climacteric were higher in the pulp. No climacteric-like changes were observed in the peel

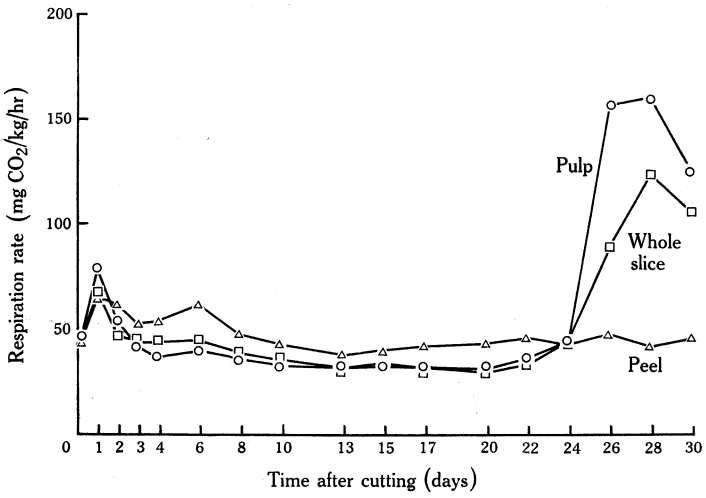


Fig. 6.—Respiration rates of peel and pulp tissue and whole slices. Each curve represents the average of three samples.

sections which were held for 30 days after cutting. These sections developed a brown discoloration after about 10 days.

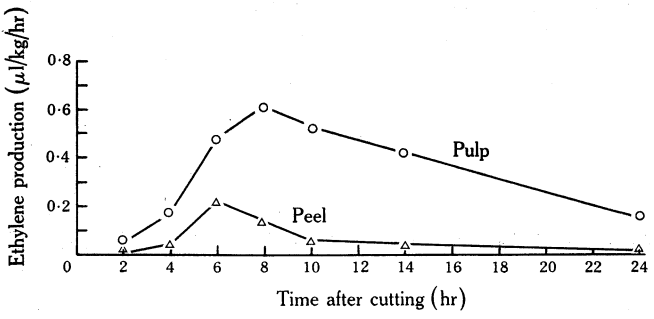


Fig. 7.—Ethylene production by peel and pulp tissue after cutting. Each curve represents the average of three samples.

(d) Effect of Ethylene Treatment on Respiration and Ethylene Production of Peel and Pulp Tissue

Sections of peel and pulp tissue were treated with 10 p.p.m. ethylene for 1 and 3 days. Both peel and pulp tissue showed an increase in respiration (Fig. 8). Pulp tissue developed a typical respiratory climacteric, but peel tissue showed a much lower peak and the rates declined within 3 days to levels normal for green tissue. Ethylene production in pulp tissue was stimulated by a 1-day treatment with ethylene (Fig. 9) but the rates were lower than during the natural climacteric, as observed in slices (Figs. 2 and 5) or whole fruit. Production by pulp tissue treated for 3 days remained at levels similar to those of untreated tissue. Treatment of peel sections for

1 or 3 days did not stimulate ethylene production and no large increases in ethylene production were observed in untreated peel sections which were held for 30 days after cutting.

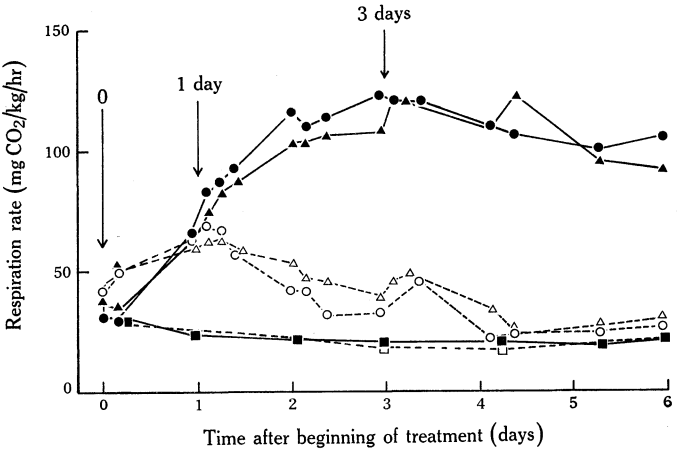


Fig. 8.—Respiration rates of peel (○,△) and pulp (●,▲) tissue treated with 10 p.p.m. ethylene for 1 (○,●) and 3 (△,▲) days. Each curve represents the average of three samples. ■ Pulp control. □ Peel control. The vertical arrows indicate the duration of ethylene treatment.

(*e*) *Effect of Ethylene Treatment on Ethylene Production Induced by Treating Slices with 2,4-D*

Four days after cutting, banana slices were infiltrated with an aqueous solution of 2,4-D (5×10^{-4}M). This treatment caused a temporary increase in respiration and ethylene production as observed when the infiltration is carried out immediately after cutting. A 24-hr application of 10 p.p.m. ethylene to 2,4-D-treated slices caused a climacteric-like rise in respiration as reported previously (Vendrell 1969). Measurements showed that the subsequent rates of endogenous ethylene production by slices treated with 2,4-D and ethylene were much lower than those of slices treated with 2,4-D alone (Fig. 10).

(*f*) *Effect of Ethylene Treatment on the Production of $^{14}C_2H_4$ by Slices Treated with [^{14}C]L-Methionine*

Burg and Clagett (1967) showed that slices of green banana fruit readily convert carbon 1 of L-methionine to CO_2 and carbons 3 and 4 to C_2H_4 but retain carbon 2 in the tissue. The present work was undertaken to obtain data to supplement the findings reported in Sections III(*a*) and III(*b*). It was postulated that if endogenous ethylene arises from methionine in banana tissue then application of ethylene should suppress the conversion of carbons 3 and 4 to ethylene with no or relatively little effect on the conversion of carbon 1 to CO_2. Analysis of the data of Table 1 showed that this was indeed the case. The probabilities for the occurrence of the decreases in the ratios of the cumulative outputs of $^{14}C_2H_4$ to $^{14}CO_2$ in the presence of 10 p.p.m.

ethylene were 0·05, 0·02, and 0·01 for 8, 12, and 24 hr respectively. These probabilities were determined by combining the probabilities for one-sided tests (Fisher 1946). The amounts of ^{14}C infiltrated that were recovered as $^{14}CO_2$ in 24 hr varied from 4 to 18% depending on the stage of ripeness of the tissue.

IV. Discussion

Ethylene treatments of sufficient duration to induce ripening clearly suppress endogenous ethylene production. This suppression is most evident immediately after ethylene treatment is stopped (Figs. 2 and 5) and although ethylene production subsequently rises it does not attain the rates observed during natural ripening. The extent of this suppression is related to the length of ethylene treatment. When the treatment is just sufficient to initiate ripening (12–15 hr) total ethylene production during the first 4–5 days of the climacteric is similar to that during natural ripening but the maximum rate is smaller and in addition there are two peaks (Fig. 2).

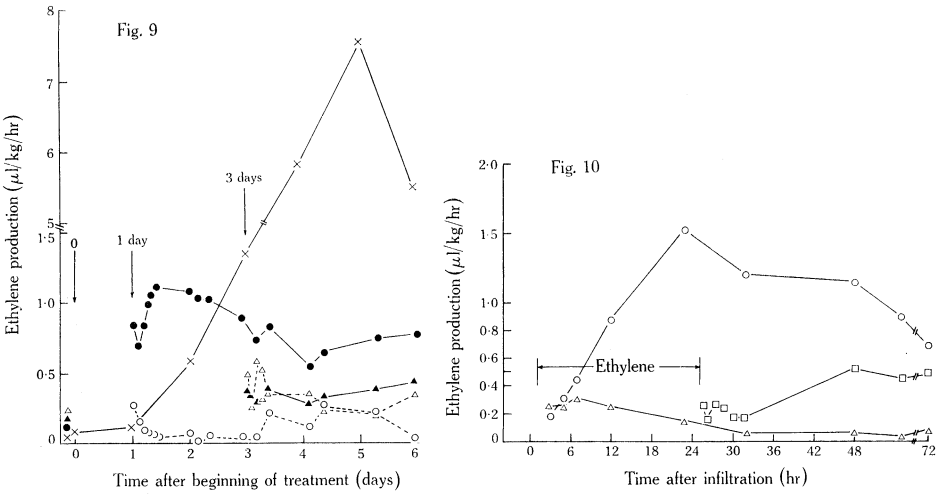


Fig. 9.—Ethylene production by peel (○,△) and pulp (●,▲) tissue after treatment for 1 (○,●) and 3 (△,▲) days with 10 p.p.m. ethylene. × Control pulp. Vacuum (66 cmHg for 1 min) was applied to remove exogenous ethylene. The data apply to the same samples referred to in Figure 8. The points at lower left are the values for ethylene production before treatment. S.E. of mean ($n' = 3$); pulp gassed 1 day, ±0·11 (32 d.f.); peel gassed 1 day, ±0·24 (30 d.f.); pulp gassed 3 days, ±0·15 (19 d.f.); peel gassed 3 days, ±0·064 (20 d.f.); untreated pulp during ripening, ±0·13 (14 d.f.). Mean of values for untreated pulp during the period of the above ethylene treatments 0·07 μl/kg/hr ($n' = 18$), S.E. of mean ±0·24 (12 d.f.). Corresponding values for untreated peel were 0·16 μl/kg/hr ($n' = 21$) and ±0·27 (14 d.f.).

Fig. 10.—Ethylene production by slices infiltrated with 2,4-D (5×10^{-4}M) and treated with 10 p.p.m. ethylene for 1 day (□). ○ Slices infiltrated with 5×10^{-4}M 2,4-D but not treated with ethylene. △ Control slices (infiltrated with water only). Other details as for Figure 1.

A possible explanation for the two peaks is that the short ethylene treatment is sufficient to trigger ripening in only a small part of the tissue. However, the rest of the tissue, being less sensitive to ethylene (Burg and Burg 1965*a*), will be activated

by the treatment and the subsequent endogenous ethylene production by the ripening tissue will be sufficient to induce ripening, giving rise to the second peak. The process, once initiated, would follow autocatalytically as in natural ripening, but be slightly more protracted. Comparative measurements of sugar content 4 days after the beginning of ethylene treatment, however, did not reveal any delay in ripening.

Figure 5 and data from other experiments show that the effects of a temporary ethylene treatment also depend on concentration up to 5–10 p.p.m. Higher concentrations do not result in greater suppression of endogenous ethylene production nor do they overcome time-dependence, although they may induce a slightly faster increase in respiration rates (Brady *et al.* 1970). The results observed in Figure 5 confirm that the inhibition of endogenous ethylene production is related to the period of treatment, even when different ethylene concentrations are used.

TABLE 1

EFFECT OF ETHYLENE TREATMENT ON THE PRODUCTION OF $^{14}C_2H_4$ BY SLICES TREATED WITH [1,2,3,4-^{14}C]METHIONINE

Three single slices were vacuum-infiltrated with aqueous solutions of [^{14}C]L-methionine (3×10^4 disintegrations/min/g, specific activity 39 mCi/mmole) at each of the times shown. The times shown for non-gassed slices are approximations based on measurements of CO_2 production. The data are presented as means of the ratios (multiplied by 1000) of the cumulative outputs of $^{14}C_2H_4$ to $^{14}CO_2$

Time after Addition of Methionine (hr)	No Ethylene Treatment: Time after Beginning of Climacteric (days)			S.E. ($n' = 6$)	Slices Treated with Ethylene: Duration of Treatment (days)			S.E. ($n' = 6$)
	0·5	1	2		0·5	1	2	
4	98	109	139	±40	51	75	141	±20
8	111	134	164	±42	40	73	121	±17
12	127	141	172	±43	41	65	120	±16
24	124	137	163	±40	35	58	110	±13

It has been reported previously that the sensitivity of banana tissue to applied ethylene increases with age after harvest (Burg and Burg 1965*a*). The present work supports this finding. The pattern of endogenous ethylene production following a 24-hr treatment of slices from a set which required 24 days to ripen naturally was nearly identical with the pattern obtained with a 12-hr treatment of slices from a set which ripened naturally in only 11 days. A similar picture is observed in fruit of about the same age, but treated with different ethylene concentrations (Figs. 2 and 5).

Chlorophyll breakdown and the magnitude of the respiratory responses require somewhat longer exposures to ethylene than does the conversion of starch to sugar [Figs. 1, 3, and 4; Section III(*a*)]. These observations tend to support earlier claims that an increase in endogenous ethylene production is a prerequisite for the initiation and continuation of the normally unified sequence of events which constitute natural ripening (Burg and Burg 1965*a*). Separated peel and pulp tissue showed different

patterns of respiration, ethylene production, and ripening. Even allowing for differences in the areas of cut surfaces, the peel produces less ethylene after cutting (Fig. 7). Production after a 1-day ethylene treatment is also lower (Fig. 9). Respiration is lower after cutting (Fig. 6) and especially during ripening induced by ethylene treatment (Fig. 8). In ethylene-treated peel sections the climacteric-like peak occurs earlier than in the pulp, and then respiration decreases to the levels of green tissue. Non-gassed sections of pulp ripen at about the same time as the whole slice or fruit, but no climacteric in respiration or other ripening changes were observed in the peel sections kept for 6 days after the pulp had commenced to ripen (Fig. 6). These observations seem to indicate that the changes in the peel observed during normal ripening depend on the high levels of ethylene production of the pulp. These findings then attach a role to the production of "excess" ethylene during natural ripening.

The inhibitory effect of added ethylene on the ethylene production induced by 2,4-D (Fig. 10) suggests that this ethylene is synthesized *via* the same pathways involved during natural ripening or ones equally susceptible to suppression by exogenous ethylene, and that the inhibitory effect does not need to be associated with ripening. Apart from stimulating respiration, 2,4-D infiltrated into banana tissue counteracts the effects of ethylene treatment and delays ripening (Vendrell 1969). Subsequently, both respiration and ethylene production decrease to the levels of control green fruit slices.

It is not known how ethylene initiates ripening (Pratt and Goeschl 1969) and consequently how exogenous ethylene suppresses endogenous ethylene production. An end-product effect could explain the low levels measured immediately after ethylene treatment, especially in treatments just sufficient to induce ripening (Figs. 2 and 5). However, the processes involved must be more complex. If an end-product type of inhibition is involved then endogenous production should rise to normal levels once exogenous ethylene is removed. Clearly the greater part of this inhibition is irreversible since the capacity to produce ethylene remains at low levels in tissue treated with 5–10 p.p.m. ethylene for 1 day or longer.

V. Acknowledgments

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