

# ACCELERATION AND DELAY OF RIPENING IN BANANA FRUIT TISSUE BY GIBBERELLIC ACID

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[*Manuscript received September 22, 1969*]

## *Summary*

Dipping of whole banana fruit in aqueous solutions of gibberellic acid ( $GA_3$ ) at concentrations of  $10^{-5}$ – $10^{-2}M$  delays ripening. In contrast, treatment of banana fruit slices by vacuum infiltration with  $GA_3$  at concentrations ranging from  $10^{-6}$  to  $10^{-2}M$  accelerates ripening. These contrasting effects appear to be related to the differences in distribution of  $GA_3$  in the tissue, resulting from the two methods of treatment.

In banana slices,  $GA_3$  slightly stimulates the rise in induced respiration and may decrease the rise in induced ethylene production initiated by cutting. Once the slices recover from these initial responses, during both the preclimacteric and climacteric periods,  $GA_3$  does not influence carbon dioxide and ethylene production. Unlike auxins,  $GA_3$  does not counteract the stimulatory effects of ethylene treatment on banana ripening.

Relative to the respiratory climacteric and the associated conversion of starch to sugar, yellowing of the skin is delayed by  $GA_3$ , whether the tissue is allowed to ripen naturally or if it is induced to ripen by ethylene treatment.

## I. INTRODUCTION

Auxins, kinins, and gibberellins have been found to regulate aging processes in many plant tissues including fruits. Auxins accelerate ripening in whole bananas (Freiberg 1955; Blake and Stevenson 1959) and pears (Hansen 1946), but in citrus fruit 2,4-dichlorophenoxyacetic acid (2,4-D) and gibberellic acid ( $GA_3$ ) retard aging of the skin and delay abscission of the calyxes (Stewart 1949; Coggins and Lewis 1962, 1965) and in tomatoes  $GA_3$ , kinetin, and indoleacetic acid (IAA) delay ripening (Abdel-Kader, Morris, and Maxie 1966). Gibberellins and kinins may also affect the metabolism of pigments in many plant tissues (Sax 1962).  $GA_3$  may cause greening in citrus fruit (Coggins and Lewis 1962) and delay the appearance of red colour in tomatoes, even when the fruit is treated with ethylene, although it does not affect the onset of the respiratory climacteric (Dostal and Leopold 1967). The presence of gibberellins or gibberellin-like substances in fruit, including bananas (Khalifah 1966) and their role in fruit setting and development have been established (Crane 1964). It seems likely that endogenous gibberellins, in conjunction with other hormones, play an important role in fruit ripening.

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Recently it was reported (Vendrell 1969) that vacuum infiltration with solutions of 2,4-D and IAA delayed ripening of banana slices, although the treatments increased the size of the peaks of respiration and ethylene production induced by cutting. The effectiveness of the auxins in delaying ripening was particularly pronounced in slices treated with 10 p.p.m. ethylene for 24 hr, which is sufficient to ripen control slices. These results which contrasted with the earlier reports that auxins accelerate ripening in whole banana fruit were attributed to the uniform distribution of the test substances achieved in the slices by vacuum infiltration (Palmer and McGlasson 1969).

In the present work, the effects of  $GA_3$  on respiration, ethylene production, and ripening in banana fruit tissue are reported.

## II. MATERIAL AND METHODS

### (a) *Source of Fruit, Preparation, and Treatment of Whole Bananas and Slices*

Bananas of the Williams Hybrid strain of the Dwarf Cavendish variety 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). Treatments were started within 5 days after harvest.

Whole bananas were treated by dipping for 30 min in sterile aqueous solutions of  $GA_3$  plus 0.025% wetting agent, then placed singly or in pairs in respiration jars and ventilated with humidified air at the rate of 0.5–1 litre/hr/banana. Bananas from the same hand were used for each set of treatments.

Immediately after cutting slices were vacuum infiltrated (66 cmHg for 1 min) with aqueous solutions of  $GA_3$ . This treatment increases the weight of slices by about 10%. Controls were infiltrated with water. Usually composite samples of four slices, cut from four matched fruit, were used. They were incubated at 20°C and ventilated with humidified air at the rate of about 1 litre/hour.

### (b) *Analyses and Ethylene Treatment*

Respiration and soluble solids were measured as described by Palmer and McGlasson (1969). Ethylene treatment (10 p.p.m. for 24 hr) has been described by Vendrell (1969) and measurement of ethylene production by McGlasson (1969).

A subjective measurement of degreening of the skin (chlorophyll breakdown) was made using the colour index devised by the Fruit Dispatch Co. (Anon. 1961). The index ranges from 1 to 8, where 1 is for green fruit and 8 for yellow fruit with large brown areas. In this work the fruit were scored only to index number 5 at which the fruit are yellow with green tips.

## III. RESULTS

### (a) *Effect on Ripening of Dipping Whole Bananas in $GA_3$ Solutions*

$GA_3$  in concentrations ranging from  $10^{-5}$  to  $10^{-2}M$  were tested. Rates of respiration and ethylene production were unaffected as was the pattern of changes in respiration or ethylene production during ripening, but the onset of the respiratory climacteric was usually delayed (Fig. 1). This delay was directly related to concentration but varied with the maturity of the fruit and the particular sample. Treatment of the fruit near the beginning of the climacteric or after it had begun had no effect.

A statistical analysis of the effects of  $GA_3$  on the delay in the onset of the respiratory climacteric was carried out on the data for 14 sets of fruit. The treatments were  $10^{-3}$  and  $10^{-5}M$   $GA_3$  and water. The time taken by control fruit to enter the respiratory climacteric ranged from 9 to 26 days, with an average of 17.7.

$10^{-3}\text{M}$   $\text{GA}_3$  caused a delay on the onset ranging from 0 to 6 days, with an average of 2.9 (16%;  $P < 0.001$ ). The effects of  $10^{-5}\text{M}$   $\text{GA}_3$  were not significant ( $P < 0.05$ ).

Ripening in treated fruit was normal as judged by the respiration curves (Fig. 1), softening of the pulp, sugar production, presence of volatiles, and ethylene production rates. However, when compared with the respiratory climacteric there was a delay in the yellowing of the skin (Fig. 2). This delay in chlorophyll breakdown was significant even when there was little or no effect on the other parameters of ripening. The delay caused by  $10^{-3}\text{M}$   $\text{GA}_3$  varied from 2 to 5 days.

Fig. 1

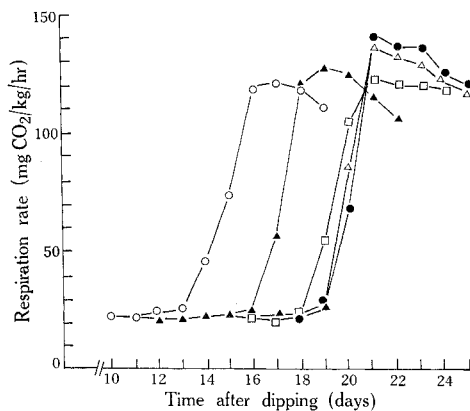


Fig. 2

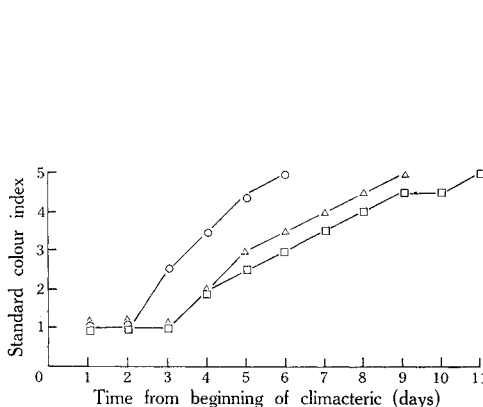


Fig. 1.—Respiration in whole bananas dipped in  $\text{GA}_3$  solutions. Two fruits were used for each treatment.  $\circ$  Control (no  $\text{GA}_3$ ).  $\bullet$   $10^{-2}\text{M}$ ,  $\triangle$   $10^{-3}\text{M}$ ,  $\square$   $10^{-4}\text{M}$ ,  $\blacktriangle$   $10^{-5}\text{M}$   $\text{GA}_3$ .

Fig. 2.—Delay in skin yellowing (standard colour index, Anon. 1961) in whole bananas dipped in  $\text{GA}_3$  solutions. Each curve represents the average of three samples.  $\circ$  Control (no  $\text{GA}_3$ ).  $\square$   $10^{-3}\text{M}$ ,  $\triangle$   $10^{-5}\text{M}$   $\text{GA}_3$ .

### (b) Effect of Vacuum Infiltration of $\text{GA}_3$ Solutions on Banana Slices

(i) *Induced Respiration and Ethylene Production.*—The peak of induced respiration due to cutting was slightly but consistently increased and delayed by  $\text{GA}_3$ , especially at high concentrations (Fig. 3). The respiration rate in treated slices subsequently declined to that of the control although high concentrations of  $\text{GA}_3$  delayed this process. In contrast,  $\text{GA}_3$  in proportion to its concentration, usually depressed the rise in induced ethylene production (Fig. 3). Ethylene production in treated slices always stabilized at low rates similar to those of the controls.

(ii) *Ripening.*—Although  $\text{GA}_3$  did not stimulate ethylene production, ripening was accelerated. This acceleration, which was indicated by the earlier onset of the climacteric, was related to concentration in the range  $10^{-6}$ – $10^{-2}\text{M}$ , and was influenced by fruit maturity (Fig. 4). In slices cut from fruit that was near the onset of the climacteric (set A) ripening was affected little, but in slices cut from less mature fruit (set B) there was a pronounced acceleration of ripening.

Measurements of ethylene production, soluble solids, softening of the pulp, and the presence of volatiles indicated that ripening in treated slices was normal, except for a delay in chlorophyll breakdown as observed in whole bananas. However, this delay was less pronounced in slices.

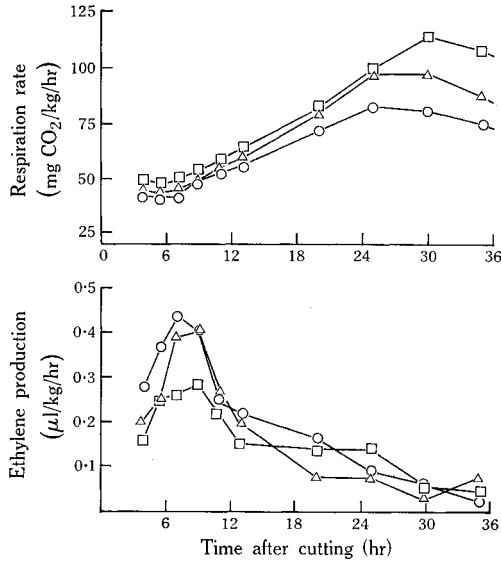


Fig. 3.—Induced respiration and ethylene production in banana slices vacuum infiltrated with GA<sub>3</sub> solutions. Each curve represents the average of three composite samples, each containing four 6-mm slices, cut from four matched fruit. ○ Control (no GA<sub>3</sub>). □ 10<sup>-3</sup>M, △ 10<sup>-5</sup>M GA<sub>3</sub>.

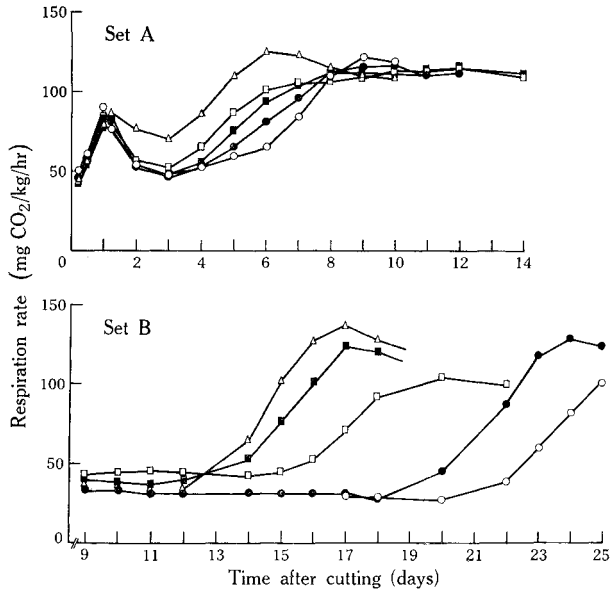


Fig. 4.—Respiration in slices vacuum infiltrated with GA<sub>3</sub> solutions from fruit of two maturities. Set A: slices cut from fruit near onset of climacteric. Set B: slices cut from less mature fruit. The peak of induced respiration that takes place after cutting is not included in set B where it is completely dissociated from the respiratory climacteric. ○ Control (no GA<sub>3</sub>). △ 10<sup>-2</sup>M, □ 10<sup>-3</sup>M, ■ 10<sup>-4</sup>M, ● 10<sup>-5</sup>M GA<sub>3</sub>.

The increase in the soluble solids content of slices during ripening was usually smaller than in whole bananas. This is probably due to an increased consumption of soluble solids which is associated with the higher respiration rates characteristic of slices. GA<sub>3</sub> did not affect the accumulation of soluble solids.

(iii) *Response to Ethylene Treatment.*—After treatment with 10 p.p.m. ethylene for 24 hr, ripening, as indicated by the onset of the respiratory climacteric and an increase in soluble solids, occurred at the same time in slices infiltrated with GA<sub>3</sub> as in controls. However, there was a delay in chlorophyll breakdown, as observed in other experiments.

#### IV. DISCUSSION

The contrasting effects of GA<sub>3</sub> on the ripening of banana tissue, depending upon whether it is applied to whole fruit by dipping, or to slices by vacuum infiltration, are similar to those obtained with 2,4-D and IAA. However, these auxins accelerate ripening in whole bananas (Freiberg 1955; Blake and Stevenson 1959) and delay ripening in slices treated by vacuum infiltration (Vendrell 1969), whereas the converse is true for GA<sub>3</sub>. It was suggested that the contrasting responses to auxins in whole fruit and slices were associated with differences in distribution obtained by the two methods of treatment. This suggestion was based on the results of previous studies which showed the poor penetration of the test substances applied to banana tissue by dipping, whereas uniform distribution is achieved by vacuum infiltration (Palmer and McGlasson 1969).

Support for this suggestion has been obtained from work with [1-<sup>14</sup>C]2,4-D (Vendrell, unpublished data) in which 24 hr after dipping whole fruit over 90% of the total radioactivity recovered from the tissue was in the skin. After 4 days the proportion in the skin had declined only to about 80%. Most of the remaining activity was found in the conducting tissue of the pulp. Negligible amounts reached the centre of the pulp or penetrated the carpels.

Thus, if the penetration by GA<sub>3</sub> in whole bananas is similar to that by 2,4-D, the different responses to GA<sub>3</sub> in whole fruit and slices could also be associated with differences in distribution. The delay of ripening in whole fruit may depend on the extent of the concentration gradient of GA<sub>3</sub> from the peel to the pulp, which is presumably established when whole bananas are dipped in solutions of GA<sub>3</sub>. No information is available on the stability of GA<sub>3</sub> in banana tissue.

One of the effects of GA<sub>3</sub> is to cause retention of chlorophyll in banana fruit and in many other green tissues (Osborne 1967). In leaves, this retention is associated with retardation of senescence, which in turn is related to a maintenance of the activity of nucleic acid and protein synthesis. Furthermore, in the delay of senescence in the Navel orange rind caused by GA<sub>3</sub> treatment, it has been suggested that this maintained synthesis keeps a more functional mitochondrial membrane (Lewis *et al.* 1967). GA<sub>3</sub> is known to induce the formation of some enzymes and the activation of others in seed tissues (Chrispeels and Varner 1967) and it is also known to increase auxin levels in other tissues (Kuraishi and Muir 1962; Valdovinos, Ernest, and Henry 1967). Thus in banana tissue GA<sub>3</sub> could act on hormonal or enzymic systems associated with the ripening process.

However, these suggestions do not explain the observation that GA<sub>3</sub> delays ripening in whole fruit, but accelerates it in slices. The auxin effects appear to indicate at least two ways of action in banana tissue. One is to stimulate endogenous ethylene production and the other is to oppose the effects of ethylene (Vendrell 1969). GA<sub>3</sub>, which in banana slices does not appreciably affect ethylene production or counteract the action of exogenous ethylene, must act in an entirely different manner. These results indicate also that there is no direct interaction between GA<sub>3</sub> and ethylene. Then, unlike 2,4-D, the contrasting effects of GA<sub>3</sub> cannot be explained by increased ethylene production. Therefore, they imply that the action of GA<sub>3</sub> is different in the pulp and the skin. It appears also that the peel plays an important role in banana ripening. It is possible that GA<sub>3</sub> in the peel induces or increases the production of a substance (or substances) that is translocated into the pulp tissue and which causes a delay in the process of ripening. This would be consistent with the observation that in banana fruit, softening of the pulp starts in the centre and proceeds outward (Simmonds 1966). The translocation of photosynthetic compounds from the peel into the pulp has been observed after treatment of bananas with <sup>14</sup>CO<sub>2</sub> (C. J. Brady, personal communication).

In fruits in general, higher concentrations of GA<sub>3</sub> are needed to produce an effect (Coggins and Lewis 1962; Abdel-Kader, Morris, and Maxie 1966; Dostal and Leopold 1967; Lewis *et al.* 1967) as compared with the amount required in other systems, such as the release of  $\alpha$ -amylase from barley endosperm, where a concentration as low as 10<sup>-11</sup>M is effective (Coombe, Cohen, and Paleg 1967). GA<sub>3</sub> is effective on ripening of banana fruit over a wide range of concentrations. Although in dipping treatments the actual concentration in the tissue is not known, in banana slices treated by vacuum infiltration the lowest concentration effective in accelerating ripening is about 10<sup>-7</sup>M, allowing for a dilution of about 10. (The treatment causes a weight increase in slices of about 10%.) These relatively high concentrations of GA<sub>3</sub> may be necessary because of the presence of higher amounts of inhibitors or the tissue being better able to metabolize GA<sub>3</sub> or both.

Little is known of the endogenous concentration of gibberellins in mature banana tissue although some work has been done with bananas at an early stage of development (Khalifah 1966). There are reports of a decline in content of endogenous gibberellins in some other fruits during maturation (Hashimoto and Rappaport 1966; Jackson and Coombe 1966) but it is difficult to think of a general decrease in the level of endogenous gibberellins in banana tissue as a cause of natural ripening in bananas, since application of GA<sub>3</sub> induces ripening in banana slices (Fig. 4). Resolution of these anomalies appears dependent on the acquisition of knowledge on the mechanism of action of GA<sub>3</sub> in banana tissue.

#### V. ACKNOWLEDGMENTS

The author wishes to thank Dr. W. B. McGlasson for helpful criticism, Mr. E. J. McMurchie for skilful technical assistance, and Mr. M. J. Franklin for preparation of the illustrations.

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