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

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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 regreening 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}M GA_3 caused a delay on the onset ranging from 0 to 6 days, with an average of 2·9 (16%; $P < 0{\cdot}001$). The effects of 10^{-5}M GA_3 were not significant ($P < 0{\cdot}05$).

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}M GA_3 varied from 2 to 5 days.

Fig. 1

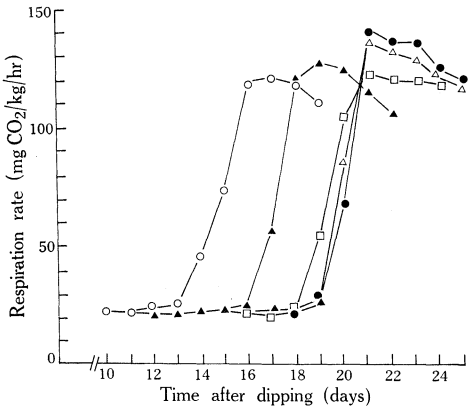

Fig. 2

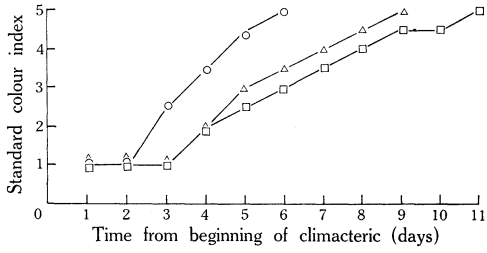

Fig. 1.—Respiration in whole bananas dipped in GA_3 solutions. Two fruits were used for each treatment. ○ Control (no GA_3). ● 10^{-2}M, △ 10^{-3}M, □ 10^{-4}M, ▲ 10^{-5}M GA_3.

Fig. 2.—Delay in skin yellowing (standard colour index, Anon. 1961) in whole bananas dipped in GA_3 solutions. Each curve represents the average of three samples. ○ Control (no GA_3). □ 10^{-3}M, △ 10^{-5}M GA_3.

(*b*) *Effect of Vacuum Infiltration of 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 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 GA_3 delayed this process. In contrast, 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 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}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.

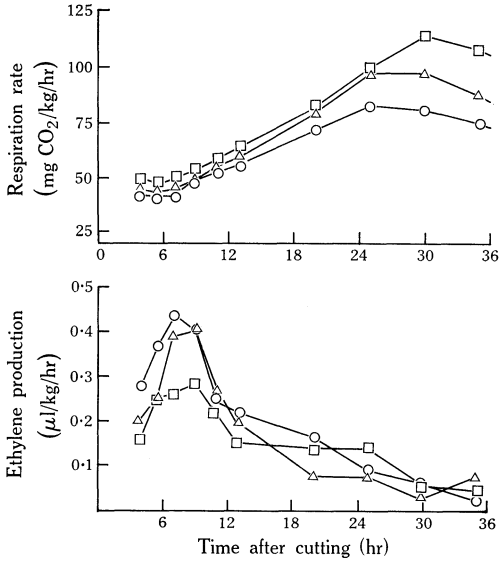

Fig. 3.—Induced respiration and ethylene production in banana slices vacuum infiltrated with GA_3 solutions. Each curve represents the average of three composite samples, each containing four 6-mm slices, cut from four matched fruit. ○ Control (no GA_3). □ 10^{-3}M, △ 10^{-5}M GA_3.

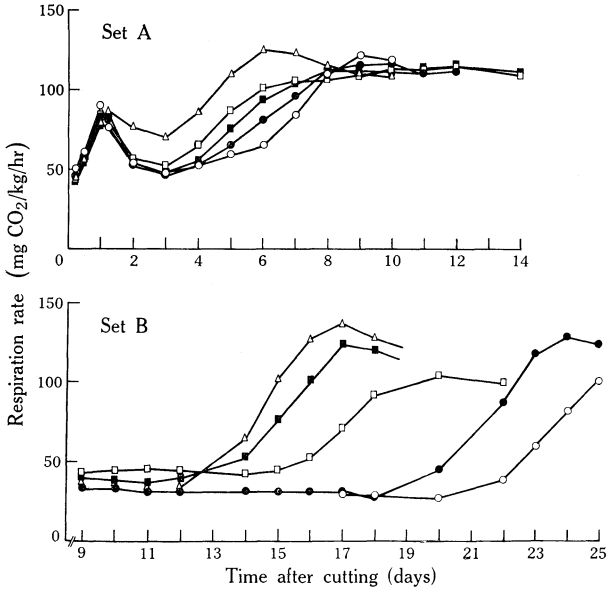

Fig. 4.—Respiration in slices vacuum infiltrated with GA_3 solutions from fruit of two maturities. Set A: slices cut from fruit near onset of climacteric. Set B: slices cut from less mature fruit. The peak of induced respiration that takes place after cutting is not included in set B where it is completely dissociated from the respiratory climacteric. ○ Control (no GA_3). △ 10^{-2}M, □ 10^{-3}M, ■ 10^{-4}M, ● 10^{-5}M GA_3.

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_3 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_3 as in controls. However, there was a delay in chlorophyll breakdown, as observed in other experiments.

IV. Discussion

The contrasting effects of GA_3 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_3. 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-^{14}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_3 in whole bananas is similar to that by 2,4-D, the different responses to GA_3 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_3 from the peel to the pulp, which is presumably established when whole bananas are dipped in solutions of GA_3. No information is available on the stability of GA_3 in banana tissue.

One of the effects of GA_3 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_3 treatment, it has been suggested that this maintained synthesis keeps a more functional mitochondrial membrane (Lewis *et al.* 1967). GA_3 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_3 could act on hormonal or enzymic systems associated with the ripening process.

However, these suggestions do not explain the observation that GA_3 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_3, 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_3 and ethylene. Then, unlike 2,4-D, the contrasting effects of GA_3 cannot be explained by increased ethylene production. Therefore, they imply that the action of GA_3 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_3 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 $^{14}CO_2$ (C. J. Brady, personal communication).

In fruits in general, higher concentrations of GA_3 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 α-amylase from barley endosperm, where a concentration as low as 10^{-11}M is effective (Coombe, Cohen, and Paleg 1967). GA_3 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^{-7}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_3 may be necessary because of the presence of higher amounts of inhibitors or the tissue being better able to metabolize GA_3 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_3 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_3 in banana tissue.

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VI. References

Abdel-Kader, A. S., Morris, L. L., and Maxie, E. C. (1966).—Effect of growth-regulating substances on the ripening and shelf-life of tomatoes. *Hortscience* **1**, 90–1.

Anon. (1961).—"Banana Ripening Manual." p. 24. (Fruit Dispatch Co.: Boston, Mass.)

Blake, J. R., and Stevenson, C. D. (1959).—Effects of growth regulating substances on the ripening of detached banana fruit. *Qd J. agric. Sci.* **16**, 87–90.

Chrispeels, M. J., and Varner, J. E. (1967).—Hormonal control of enzyme synthesis: On the mode of action of gibberellic acid and abscisin in aleurone layers of barley. *Pl. Physiol., Lancaster* **42**, 1008–16.

Coggins, C. W., Jr., and Lewis, L. N. (1962).—Regreening of Valencia orange as influenced by potassium gibberellate. *Pl. Physiol., Lancaster* **37**, 625–7.

Coggins, C. W., Jr., and Lewis, L. N. (1965).—Some physical properties of the navel orange rind as related to ripening and to gibberellic acid treatments. *Proc. Am. Soc. hort. Sci.* **86**, 272–9.

Coombe, B. G., Cohen, D., and Paleg, L. G. (1967).—Barley endosperm bioassay for gibberellins. I. Parameters of the response system. *Pl. Physiol., Lancaster* **42**, 105–12.

Crane, J. C. (1964).—Growth substances in fruit setting and development. *A. Rev. Pl. Physiol.* **15**, 303–26.

Dostal, H. C., and Leopold, A. C. (1967).—Gibberellin delays ripening of tomatoes. *Science, N.Y.* **158**, 1579–80.

Freiberg, S. R. (1955).—Effect of growth regulators on ripening, split peel, reducing sugars, and diastatic activity of bananas. *Bot. Gaz.* **117**, 113–19.

Hansen, E. (1946).—Effect of 2,4-dichlorophenoxyacetic acid on the ripening of Bartlett pears. *Pl. Physiol., Lancaster* **21**, 588–92.

Hashimoto, T., and Rappaport, L. (1966).—Variations in endogenous gibberellins in developing bean seeds. I. Occurrence of neutral and acidic substances. *Pl. Physiol., Lancaster* **41**, 623–8.

Jackson, D. I., and Coombe, B. G. (1966).—Gibberellin-like substances in the developing apricot fruit. *Science, N.Y.* **154**, 277–8.

Khalifah, R. A. (1966).—Gibberellin-like substances from the developing banana fruit. *Pl. Physiol., Lancaster* **41**, 771–3.

Kuraishi, S., and Muir, R. M. (1962).—Increase in diffusible auxin after treatment with gibberellin. *Science, N.Y.* **137**, 760–1.

Lewis, L. N., Coggins, C. W., Jr., Labanauskas, C. K., and Dugger, W. M., Jr. (1967).—Biochemical changes associated with natural and gibberellin A_3 delayed senescence in the Navel orange rind. *Pl. Cell Physiol. Tokyo*, **8**, 151–60.

McGlasson, W. B. (1969).—Ethylene production by slices of green banana fruit and potato tuber tissue during the development of induced respiration. *Aust. J. biol. Sci.* **22**, 489–91.

Osborne, D. J. (1967).—Hormonal regulation of leaf senescence. *Symp. Soc. exp. Biol.* **21**, 305–21.

Palmer, J. K., and McGlasson, W. B. (1969).—Respiration and ripening of banana fruit slices. *Aust. J. biol. Sci.* **22**, 87–99.

Sax, K. (1962).—Aspects of aging in plants. *A. Rev. Pl. Physiol.* **13**, 489–506.

Simmonds, N. W. (1966).—"Bananas." p. 219. (Longmans, Green, & Co. Ltd.: London.)

Stewart, W. S. (1949).—Effects of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid on citrus fruit storage. *Proc. Am. Soc. hort. Sci.* **54**, 109–17.

Valdovinos, J. G., Ernest, L. C., and Henry, E. W. (1967).—Effect of ethylene and gibberellic acid on auxin synthesis in plant tissues. *Pl. Physiol., Lancaster* **42**, 1803–6.

Vendrell, M. (1969).—Reversion of senescence: effects of 2,4-dichlorophenoxyacetic acid and indoleacetic acid on respiration, ethylene production, and ripening of banana fruit slices. *Aust. J. biol. Sci.* **22**, 601–10.