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# Influence of Plant Hormones on Ethylene Production in Apple, Tomato, and Avocado Slices during Maturation and Senescence

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## ABSTRACT

**Ethylene production by tissue slices from preclimacteric, climacteric, and postclimacteric apples was significantly reduced by isopentenyl adenosine (IPA), and by mixtures of IPA and indoleacetic acid, and of IPA, indoleacetic acid, and gibberellic acid after 4 hours of incubation. Ethylene production by apple (*Pyrus malus* L.) slices in abscisic acid was increased in preclimacteric tissues, decreased in climacteric peak tissues, and little affected in postclimacteric tissues. Indoleacetic acid suppressed ethylene production in tissues from preclimacteric apples but stimulated ethylene production in late climacteric rise, climacteric, and postclimacteric tissue slices. Gibberellic acid had less influence in suppressing ethylene production in preclimacteric peak tissue, and little influenced the production in late climacteric rise, climacteric peak, and postclimacteric tissues. IPA also suppressed ethylene production in pre- and postclimacteric tissue of tomatoes (*Lycopersicon esculentum*) and avocados (*Persea gratissima*). If ethylene production in tissue slices of ripening fruits is an index of aging, then IPA would appear to retard aging in ripening fruit, just as other cytokinins appear to retard aging in senescent leaf tissue.**

Ripening and aging of fruits are associated with their production of ethylene. Ethylene is also assumed to be the hormone which initiates the ripening process (1). Since some fruits ripen without apparent involvement of ethylene, its role as the major fruit ripening agent has been questioned (2, 9). Other plant hormones have been suggested as being involved, with ethylene, in the ripening and senescence of fruit (2). Interaction between ethylene and other plant hormones was observed in seedlings, wherein auxins induced ethylene production and cytokinins and gibberellins influenced either ethylene production or action (5, 6). The interrelationships among hormones in ripening and senescing fruits are less clear and may be more subtle (2), but fruit ripening most likely involves interactions between ethylene and other plant hormones.

We report herein the influence of various hormones on ethylene production in slices from preclimacteric, climacteric, and postclimacteric apples, tomatoes, and avocados. This information should help clarify the possible interrelationships between ethylene and other plant hormones in ripening and in aging fruit tissues.

## MATERIALS AND METHODS

Golden Delicious apples (*Pyrus malus* L. cv. Golden Delicious) grown at Beltsville were selected from a single tree. Experiments with preclimacteric fruit were carried out with fruit picked from the tree in early morning before each experiment. Fruit harvested in late September were stored at 0 C and withdrawn periodically for experiments through January. Upon removal from cold storage, a sample of five fruit was brought to room temperature and then sampled at 20 C for ethylene production. Another five fruit were cut into slices 0.5-cm thick from which discs of 1 cm diameter were cut with a corkborer. The discs were washed in 0.4 M sucrose, and then incubated in 25-ml flasks containing various hormones in 0.4 M sucrose. The possibility that microbial contamination would influence ethylene production was tested by addition of 100 $\mu$g chloramphenicol/flask. Since the presence of chloramphenicol did not alter ethylene production, it was included in the incubation solutions. The solutions contained 0.1 mM IAA, IPA,[2] $GA_3$, ABA, a combination of IAA and IPA, or a combination of IAA, IPA, and $GA_3$. The concentrations of hormones used were in line with those used with fruit tissues in other studies (4, 11). Each incubation flask contained four apple slices (about 1 g), 5 ml solution, and a vial of 20% KOH to absorb $CO_2$. The flasks were sealed with serum caps and incubated at 30 C in a shaker bath. The atmosphere within each flask was sampled 4, 8, 12, and 24 hr after capping. The serum cap was removed after each sampling and the flask atmosphere blown out with air.

Tomato (*Lycopersicon esculentum*, Mill.) slices (2 g) were prepared as previously described (7) and were incubated in 3 ml of 0.1 mM hormonal solutions that were 0.028 M citrate, 0.047 M phosphate, and 0.4 M sucrose (pH 4.6). Mature green fruit were ripened at 15 C and sampled in the pre- and postclimacteric stages. Avocado (*Persea gratissima*, Gaertn., cv. Fuerte) fruit, obtained by air freight from California in the mature green preclimacteric stage, were sampled immediately and again after ripening at 15 C in the postclimacteric condition. Discs of avocado tissue were prepared like those of apple tissue but were 0.3 cm thick. They were incubated in 0.1 mM hormonal solutions that were 0.4 M sucrose, 0.028 M citrate, and 0.047 M phosphate buffer (pH 4.6), and were sampled periodically for ethylene production.

Sampling of the atmosphere was carried out by gas-tight hypodermic syringe. Ethylene was determined by gas chromatography with a sensitive electrometer-flame ionization detector and an alumina column held at 35 C, as previously described (10). Each treatment was tested in triplicate and each experiment was repeated at least three times. The data reported are averages of three experiments for each hormone treatment and control. The standard deviations of the means were generally about 10 to 15% of the mean values. The data were submitted to analysis of variance and statistical significance was found for virtually all of

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[2] Abbreviation: IPA: isopentenyl adenosine.

the IPA treatments and some of the IAA treatments as indicated under "Results."

## RESULTS

**Ethylene Production of Whole Apples during Senescence and Ripening.** Ethylene production of intact apples from early September to January indicated that the apples were in the preclimacteric stage at the start of these experiments. With increased time in 0 C storage, the apples showed the usual rise and fall of ethylene production associated with the climacteric (Fig. 1). Ethylene production increased noticeably a few days after the fruit were placed in cold storage, then rose sharply until it peaked about 9 weeks after harvest. Thirteen weeks after harvest the fruit were well beyond the climacteric peak.

**Effect of Hormones on Ethylene Production in Preclimacteric Apple Slices.** Slices prepared from preclimacteric fruit started to produce measurable amounts of ethylene after an initial 4-hr incubation period reaching a peak at 12 hr of incubation, regardless of treatment (Fig. 2). Rates of ethylene production then declined in all treatments except in ABA and control. At 12 hr of incubation, the rates of ethylene production, as compared to controls, were greater for the ABA treatment, less by 30% for the IAA treatment, and less by 57% for the treatments that included both IAA and IPA. During 12 to 24 hr of incubation, the rate of ethylene production compared to controls was about 25% greater in ABA, and decreased somewhat (17%) in GA. In this incubation period, significant reduction in ethylene production, compared to control, occurred in tissue slices incubated in either IAA or IPA (about 50%) and in combination of IAA and IPA, and IAA, IPA, and GA (about 85%) (Fig. 2).

**Effect of Hormones on Ethylene Production in Early Climacteric Apple Slices.** Tissues from early climacteric fruit showed a rise and fall in ethylene production with time, regardless of the solutions in which they were incubated (Fig. 2). The rate of ethylene production peaked at 12 hr of incubation. Levels of ethylene production were much higher and the declines in ethylene production after 12 hr of incubation were generally sharper in these tissues than in preclimacteric apple tissues. Compared to controls, tissues in ABA had higher rates of ethylene production between 12 and 24 hr of incubation. Up to 12 hr, the rates of ethylene production were greater for the GA treatment than for the control but thereafter, the rates were about equal for both treatments. Tissues in IAA evolved the same levels of ethylene as controls throughout. On the other hand, IPA suppressed the rate of ethylene production by about 20% at the end of 24 hr of incubation, but had little influence during the first 12 hr. When mixtures of IAA, IPA, and GA were used, the rate of ethylene production was suppressed 40 to 50% at 8 hr and about 50% at the end of 24 hr of incubation.

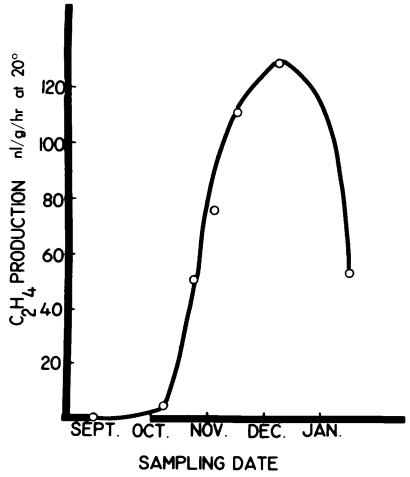


FIG. 1. Ethylene production by intact Golden Delicious Apples prior to storage and periodically after removal from 0 C storage and warming to 20 C for assay.

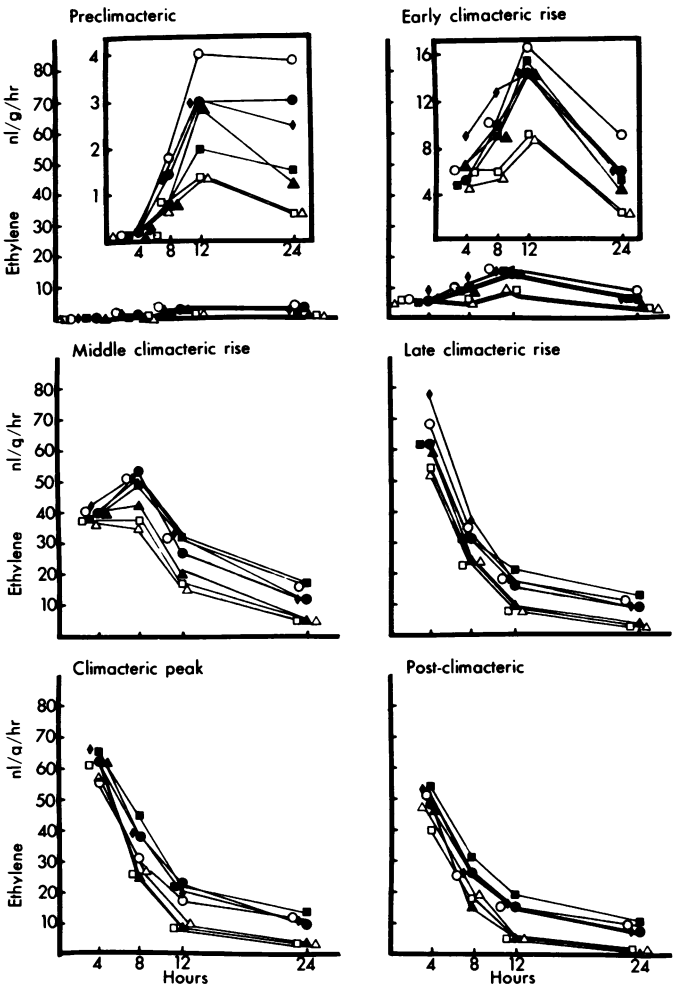


FIG. 2. Rates of ethylene production by tissue; slices from apples at different stages of ripeness during incubation for 24 hr in solutions containing 0.4 M sucrose and 0.1 mM hormones: ●: control (no hormones); ■: IAA; ◆: GA; ▲: IPA; ○: ABA; □: IAA + IPA; △: IAA + IPA + GA.

**Effect of Hormones on Ethylene Production in Midclimacteric Apple Slices.** The curves for rates of ethylene production in slices from midclimacteric apples differed from those for early climacteric fruit. In addition to much greater ethylene production, the rise was small and the decline started earlier after peaking at 8 hr (Fig. 2). Tissues in IPA showed only a slight rise, whereas those in the combination treatments showed no rise at all. During the first 12 hr, the rates of ethylene production for the ABA, IAA, and GA treatments were similar to those for the control. In the 12- to 24-hr period, the rates for the IAA and ABA treatments were about 35% higher than the rate for control. Tissues in IPA and in the hormone mixtures consistently showed much lower rates (about 50%) of ethylene production than control tissues especially after 4 hr of incubation (Fig. 2).

**Effect of Hormones on Ethylene Production by Apple Slices in Late Climacteric, Climacteric Peak, and Postclimacteric Stages.** In contrast to tissues from younger fruit, tissues from older fruit showed no increase or peak in their ethylene production. Rates of ethylene production declined steadily throughout the 24-hr incubation period, regardless of the treatment, in tissue from late climacteric rise, climacteric peak, and postcli-

macteric fruit (Fig. 2). Generally ABA and GA had little influence on ethylene production compared to controls, in these older tissues, especially in the 12- to 24-hr incubation period. In the first 8 hr of incubation, ABA and GA increased ethylene production about 10 to 20% in late climacteric tissue and ABA decreased ethylene production about 10 to 20% during the first 12 hr in climacteric peak tissue. These differences may indicate a trend but are not statistically significant. Except for the 12-hr climacteric tissue, IAA increased ethylene production in all older tissues after the first 8 hr of incubation and these differences were statistically significant. Ethylene production increased 30 to 40% in most of these tissues during the 12- to 24-hr period in IAA. The cytokinin, IPA, increasingly and consistently suppressed ethylene production 5 to 56% in these older tissues after the first 4 hr and as high as 75% in postclimacteric tissue at 24 hr. Hormone mixtures during the first 4 hr of incubation suppressed ethylene production much more than IPA did. However, after 4 hr incubation the hormone mixtures or IPA were equally effective in decreasing ethylene production (Fig. 2). This contrasts with the influence of IPA on ethylene production in preclimacteric tissues, wherein IAA and GA accentuated the suppressive influence of IPA alone. Analysis of variance verified that all of the data for treatments with IPA except for 4-hr incubation periods were significantly different from those for the controls throughout these experiments with apple slices.

**Effect of Hormones on Ethylene Production in Ripening Tomato and Avocado Slices.** IAA did not influence ethylene production greatly (about 11% decrease) in preclimacteric tomato slices (green fruit) during 26 hr of incubation at 25 C (Fig. 3). On the other hand, IPA and the combination of IPA and IAA reduced the rate of ethylene production about 55 and 48%, respectively, by the end of 26 hr. IAA appeared to counteract partly the inhibition of ethylene production by IPA.

When tissue from tomatoes at the climacteric peak (pink fruit) were incubated in IAA, after 18 hr they produced ethylene at a rate about 30% greater than controls (Fig. 3). IPA suppressed the rate of ethylene production about 32%, but the combination of IPA and IAA suppressed the rate only 17%. This again suggests a tendency of IAA to antagonize or oppose the suppressive action of IPA on ethylene production in tomato slices.

IAA stimulated the rate of ethylene production in green preclimacteric avocado tissues by 15% in 10 hr of incubation (Fig. 4). IPA suppressed ethylene production in these tissues about 40% at 10 hr of incubation. A combination of IPA and IAA reduced ethylene production about 27% after 10 hr of incubation.

Ethylene production in postclimacteric avocado tissues was reduced somewhat (10%) by IAA after 9 hr of incubation (Fig. 4). IPA and kinetin suppressed ethylene production about 57 and 47%, respectively, by the end of 9 hr. The combination of IPA and IAA reduced ethylene production 50% by the end of this incubation period.

## DISCUSSION

The curves for ethylene production shown by apple slices of various ages incubated for 24 hr at 30 C were reminiscent of the climacteric curve associated with ripening and aging of intact apples. The incubation period, therefore, appeared to age the tissue slices much as the storage period aged the intact fruit, at least with respect to ethylene production. The rise and fall in ethylene production in whole fruits are phenomena associated with aging and senescence. Consequently, the ability of hormones to alter ethylene production could be viewed as their ability to alter the aging process. Furthermore, the spectrum of aging states in the fruit may be represented by levels of ethylene production during the preclimacteric and various stages of the climacteric. If the growth hormones (auxins, GA, and cytokinins) can suppress aging, they will likely be most effective in the preclimacteric stage, before reactions associated with aging are fully set in motion. Indeed, all growth hormones (IAA, GA, IPA) suppressed ethylene production in tissues from preclimacteric apples. Suppression was greater by the mixtures of growth hormones (about 75%) than by IAA or IPA alone (about 50%). ABA, on the other hand, stimulated ethylene production in these preclimacteric tissues.

The response of the slices to hormones varied with the age of

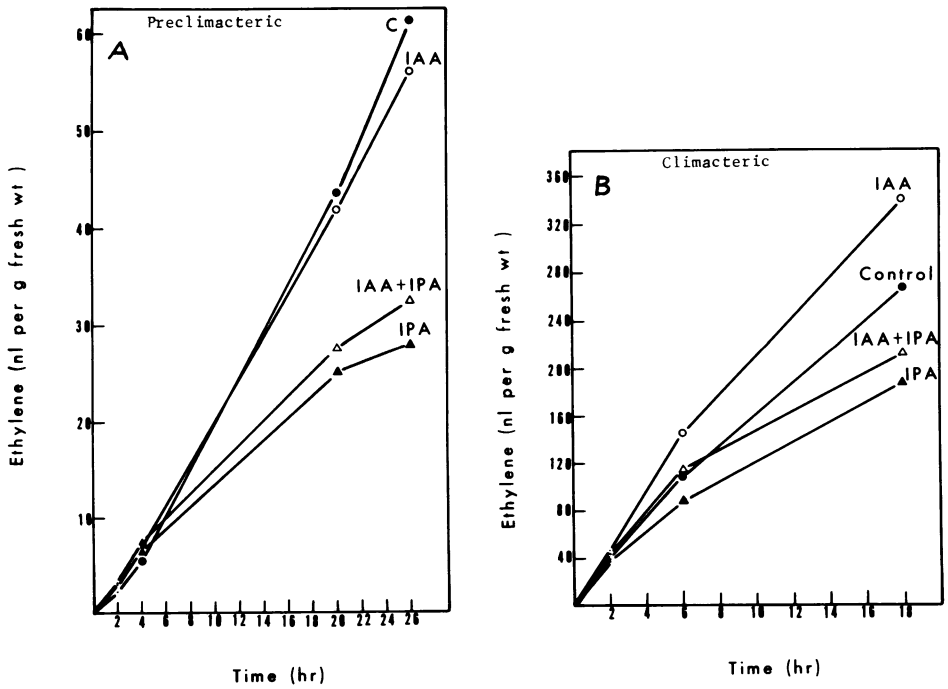


FIG. 3. Ethylene production by tissue slices of preclimacteric and climacteric tomatoes treated with various hormones (0.1 mM).

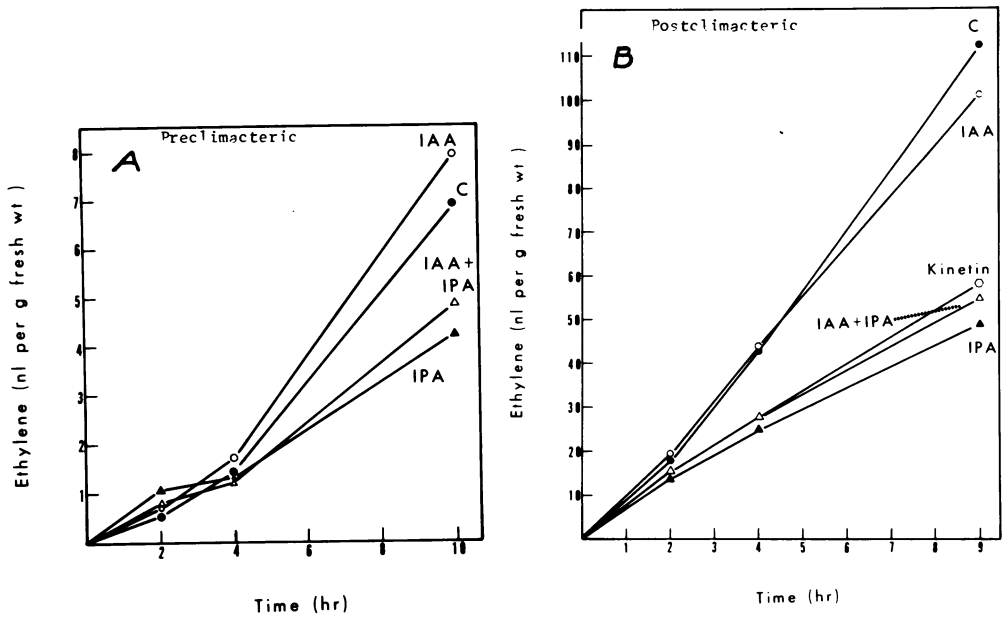


FIG. 4. Ethylene production by tissue slices of preclimacteric and postclimacteric avocados treated with various hormones (0.1 mM).

the apples. The effect of GA on ethylene production after the preclimacteric was marginal, which may indicate that GA has little influence on the aging process during the climacteric rise and fall in apple tissue. ABA stimulated ethylene production slightly but consistently in tissues not yet at their climacteric peak. With tissues at the climacteric peak, ABA appears to suppress ethylene production about 10%. This may further indicate that ABA accelerates aging (3), since rates of ethylene production decline in apples that are beyond the climacteric peak. Also, the consistent low level stimulation of ethylene production by ABA before the climacteric peak suggests that it accelerates aging in these tissues.

The stimulative effect of IAA on ethylene production in tissues from apples beyond the early climacteric rise may not represent an acceleration of aging but rather a specific response of the tissue to high superphysiological levels of auxin. The mechanism of this stimulation by IAA may be analogous to its stimulation of ethylene production in etiolated pea seedlings (8). There is evidence that IAA tends to counteract the aging process in some fruit tissues on the climacteric rise and in postclimacteric stages of senescence, despite its stimulation of ethylene production. Vendrell (14) demonstrated this in banana and Frenkel and Dyck (4) in pears. In avocado, delay of ripening with auxin at much lower concentration levels than the level we used was not accompanied by increased ethylene production (13). These experiments (4, 13, 14) and ours suggest that auxins can delay both ripening and ethylene production during the preclimacteric, and delay senescence in climacteric tissue even though ethylene is increased.

The potent and consistent suppressive action of the cytokinin, IPA, on ethylene production in all stages of ripening and senescence, of the three fruit tissues studied, bears on the question of whether or not ethylene may be a causative agent in senescence and ripening of fruit (2, 9). The fact that IPA is most effective in the 12- to 24-hr period of incubation, when senescence is rapidly accelerating, further suggests the antisenescence activity of IPA. Cytokinins have been shown to oppose aging in leaf tissues by preserving protein synthesis and Chl (12); in tomato fruit without influencing ethylene production (11); and in banana even in the presence of ethylene (15). The evidence for considering ethylene the causative agent in fruit ripening has been documented by Burg (1). Our experiments further indicate that IPA suppressed ethylene production in fruit tissues throughout the various stages of ripening and senescence, suggesting a physiological antagonism between these two hormones. On the other hand, infiltration of cytokinin into preclimacteric avocado fruit had virtually no effect on rate of ripening or ethylene production (13).

Although we have correlated tissue treatments with single hormones and their combinations in tissue slices, we are aware that we were not dealing with intact fruit and that ripening and senescence are complex and subtle phenomena. Nevertheless, we believe, there is sufficient evidence available for us to conclude that ethylene is not the sole arbiter of aging in fruit and other plant tissues. Auxins and cytokinins appear to play significant roles in the control of ethylene production by plant tissues. However, their influence on ethylene production may not always be directly correlated with aging in fruit tissues. Clarification of individual and combined roles of these hormones in aging will depend on elucidation of their general mechanisms of action.

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