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Promotion of Softening Processes in Pear by Acetaldehyde, Independent of Ethylene Action[1]

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Additional index words. acetaldehyde, ethylene, ripening

Abstract. **Acetaldehyde is produced in fruit of pear (*Pyrus communis* L.) and can stimulate ripening. The action of selective inhibitors indicates that acetaldehyde operates independently of ethylene.**

Fidler (6) has demonstrated that acetaldehyde can stimulate respiration in fruit and we have shown (9) that the effect of acetaldehyde on respiration in blueberry and strawberry is apparently independent of ethylene action. The purpose of this study was to evaluate the effect of acetaldehyde on pear ripening and to determine if the application of acetaldehyde could overcome the inhibitory effect of certain ethylene antagonists.

Materials and Methods

'Bosc' pears from Pine Grove, Pa. were stored as previously described (10).

Acetaldehyde was applied to fruit in which the action or the synthesis of ethylene was blocked. This was accomplished by either storing the fruit under hypobaric conditions or by storing the fruit under normal atmospheric conditions and applying certain inhibitory compounds. All experiments were conducted at room temp ($22^{\circ} \pm 2^{\circ}$C). Hypobaric conditions were used for the rapid removal and the inhibition of ethylene action in pear. The fruit was stored in hypobaric chambers (7) and kept at 1/10th atmospheric tension, thus allowing for an O_2 tension equivalent to 10% O_2. Oxygen (100%) was continuously flowing through the chambers at a rate of 400 ml/min which allowed one change of the chamber atmosphere every 4 hr. The effect of ethylene was also blocked by the application of alpha, alpha-dipyridyl, and silver nitrate, as described previously (10). Fruits treated with alpha, alpha-dipyridyl or silver nitrate were kept in glass chambers and ventilated with air at a rate of 400 ml/min.

Acetaldehyde was applied to the fruit in the ventilating gases. The concn of the volatile was regulated by mixing a source gas mixture of acetaldehyde of a known concn with the ventilating gas, as described before (9).

Changes in acetaldehyde and ethanol, which occur naturally in ripening pears, were determined according to method of Davis and Chace (5). Tissue increments weighing 50 g each were removed periodically from intact ripening pears and homogenized in 100 ml of trichloroacetic acid at -5° to -10°C. A 40 ml aliquot of the homogenate was placed in 125 ml serum bottles, which were incubated in a water bath at 37° for 1 hr. After incubation, 1 ml samples were drawn from the head space and used for determining acetaldehyde and ethanol by gas chromatography.

Ethylene and CO_2 evolution were measured daily as described previously (10). Softening was measured at 2-day intervals.

Results

Fig. 1 shows the changes in ethanol and acetaldehyde that occur normally during the ripening of pears. Ripening fruit show increased production of acetaldehyde and ethanol, representing glycolytic end products. At the early stages of ripening, production of acetaldehyde predominates, though ethanol increases markedly at the later ripening stages. At the peak of formation, the ethanol was roughly 10-fold the level of the acetaldehyde. This is consistent with data reported previously (6). The application of acetaldehyde under standard conditions increased the softening in pear and the ethylene

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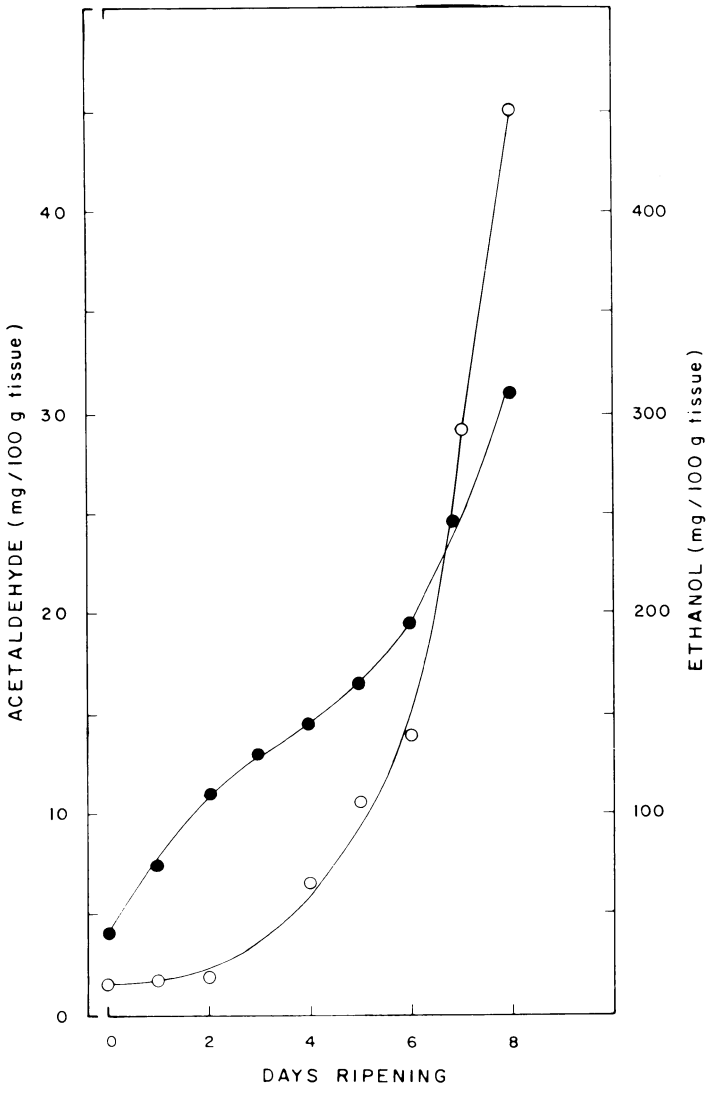

Fig. 1. The production of acetaldehyde (•) and ethanol (○) during ripening of 'Bosc' pears.

production of the tissue (Fig. 2). The effect of acetaldehyde showed a characteristic dose-response. Ethylene production and softening were promoted progressively as the concn increased from 300 to 1300 μl/liter, but were inhibited at a high concn (3000 μl/liter). Because ethylene synthesis was also promoted, it is possible that the observed stimulation of softening by acetaldehyde represented an indirect ethylene effect. The following experiments were conducted to delineate the effects of ethylene apart from those of acetaldehyde, in order to establish if the latter can function independently.

In one approach we employed hypobaric storage, which is used to acelerate the escape of the endogenous ethylene from tissues and thereby retard ethylene-dependent processes, such as fruit ripening. Under hypobaric conditions, ethylene synthesis conditions, presumably reflecting a response to stress conditions (Fig. 3). Even though ethylene synthesis was accelerated, however, there was a delay in softening, possibly because the action of ethylene under these conditions was curtailed. Adding acetaldehyde to the storage atmosphere overcame the inhibition of softening by the hypobaric conditions, and resulted in an acceleration of the softening process. The resulting softening pattern could not be attributed to the action of ethylene. When ethylene production was accelerated, softening was delayed; conversely, deferring the synthesis of ethylene, as induced by acetaldehyde, was associated with accelerated softening. The rate of softening could be correlated with the effect of acetaldehyde, however. When native volatiles were removed, at low atmospheric tensions, softening was inhibited. Upon adding acetaldehyde, the inhibition was overcome and softening was accelerated. For these reasons, we suggest that the promotive action of acetaldehyde was not an indirect effect of ethylene.

To further differentiate between the effects of ethylene and acetaldehyde, we chose to antagonize the action of ethylene in situ by applying alpha, alpha-dipyridyl and silver nitrate. We have shown (10) that alpha, alpha-dipyridyl can be used to inhibit fruit softening, presumably by inhibiting the ethylene-stimulated, cyanide-resistant respiration (1). Silver, the ethylene antagonist (2), likewise inhibits softening in pear. In the present work we used these compounds to inhibit the action of ethylene and to examine how the treated fruit will respond to acetaldehyde. The effect of ethylene and acetaldehyde on softening in control fruit (infiltrated with mannitol), as compared to fruit infiltrated with mannitol plus alpha, alpha-dipyridyl is shown in Fig. 4. Both volatiles accelerated the softening process in mannitol infiltrated fruit. In the pears treated with alpha, alpha-dipyridyl, however, ethylene did not overcome the inhibitory effect of the compound. By comparison, acetaldehyde stimulated softening, approaching the rate of the control.

In a similar experiment (Fig. 5) we showed that silver nitrate, the ethylene antagonist, markedly inhibited softening. As with the dipyridyl compound, ethylene did not overcome the inhibitory effect of the silver, and by comparison acetaldehyde showed the ability to induce increased softening.

Discussion

The results presented indicate that acetaldehyde occurs normally in fruit and builds up during the ripening process. Applications of acetaldehyde induced an increased rate of ripening in 'Bosc' pear. Since acetaldehyde stimulated ethylene production (Fig. 1) it's effect on softening may be an indirect ethylene effect, therefore, the role of acetaldehyde had to be studied as related to the metabolism of ethylene. It has also been shown in apple labelled acetaldehyde could be converted to ethylene (11), supporting the idea of an ethylene involvement in the acetaldehyde induced acceleration of ripening. This speculation was tested by removing ethylene under hypobaric conditions, by inhibiting ethylene metabolism with the silver ion, and alpha, alpha-dipyridyl.

Our results indicate that acetaldehyde caused increased softening in fruit when ethylene was removed rapidly in hypobaric storage, suggesting that the action of acetaldehyde was not an indirect ethylene effect. It is still possible, however, that acetaldehyde was increasing the tissue sensitivity to ethylene, and therefore, was allowing an increased response to even low levels of ethylene. Also acetaldehyde has been shown to be a possible substrate for ethylene production. Therefore, to further demonstrate whether acetaldehyde acts through ethylene or not it became necessary to interfere with the metabolism of ethylene. We used silver and the dipyridyl compound to antagonize the action of ethylene. The ability of acetaldehyde to overcome, at least in part, the inhibition of the ethylene antagonists indicates that the action of acetaldehyde was not an indirect effect of ethylene.

It appears, therefore, that acetaldehyde contributed to the overall stimulation of ripening processes independently of ethylene. From the standpoint of concn, acetaldehyde seemed to be much less efficient than ethylene; that is acetaldehyde was used at a concn of 1,300 μl/liter to maximally promote ripening, while ethylene could do so at a concn of 10 μl/liter. The acetaldehyde concn used in this study, although rather

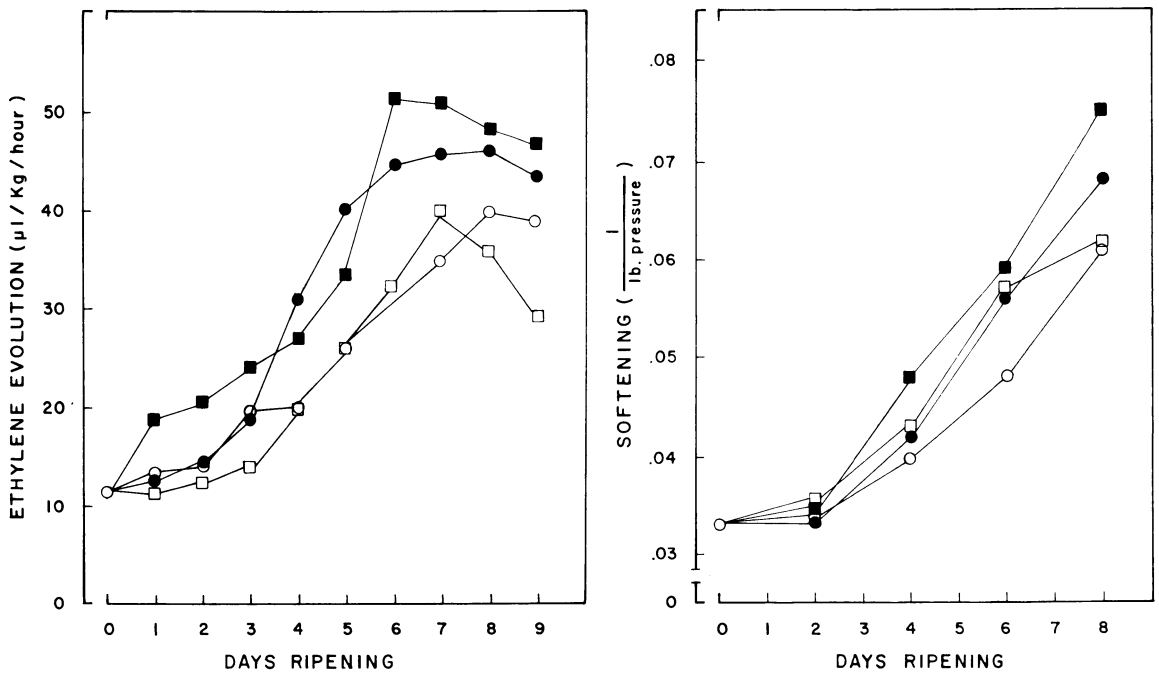

Fig. 2. The effect of various concn of acetaldehyde (zero µl/liter (○), 300 µl/liter (●), 1,300 µl/liter (■), 3,000 µl/liter (□)) on ethylene synthesis and softening in pears.

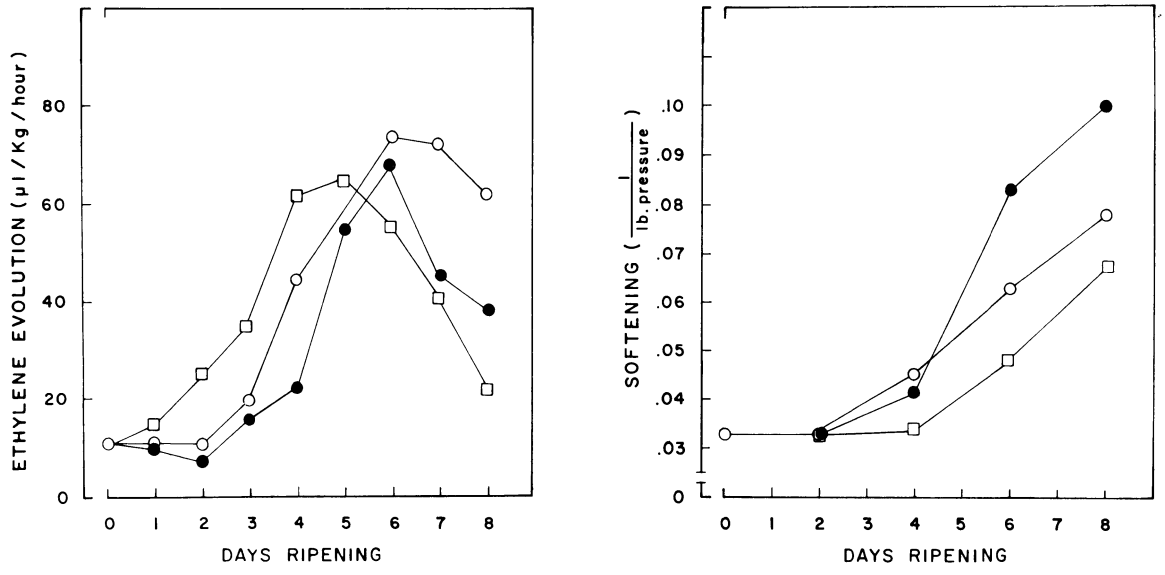

Fig. 3. Softening and ethylene synthesis in pears under standard atmospheric conditions (○) and in hypobaric storage in the presence (●) and the abscence (□) of acetaldehyde (1,300 µl/liter).

high by comparison to the effective ethylene concn, were in the range of those that are produced as the pear fruit ripens. It appears that acetaldehyde may be one of the factors contributing to ripening and may be important in tissues such as pear where it is produced in large quantities. The importance is not so much the magnitude of acetaldehyde's contribution but that it does contribute and that it does so independently of ethylene.

At present it is not known whether the action of acetaldehyde reflects the utilization of the compound or the stimulation of metabolic processes leading to ripening. The compound has been shown to influence processes such as the Krebs cycle, electron transport, and lipid metabolism (3, 4). Fidler has also indicated (6) that acetaldehyde was metabolized by apple fruit. It is possible, therefore, that some product resulting from the metabolism of acetaldehyde may be responsible for some of its activity. Fidler's work differs from ours, however, in that he was unable to detect increased softening in apple treated with acetaldehyde, although the pear responded differently. The metabolsim and the action of acetaldehyde may be important in the overall regulation of the ripening process, and in metabolic disorders that often accompany

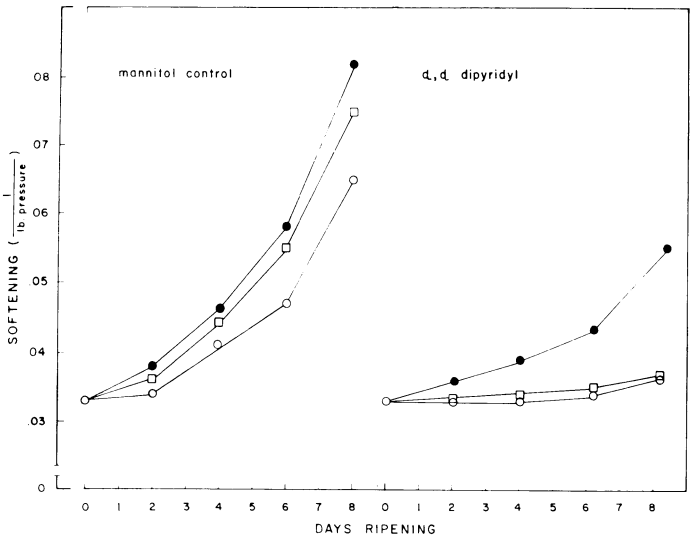

Fig. 4. The softening of pears infiltrated with carrier mannitol solution (0.3 M) and mannitol plus 10^{-2} M alpha, alpha-dipyridyl as occurring in air (○) and as effected by 10 μl/liter ethylene (□) , or 1,300 μl/liter acetaldehyde (●).

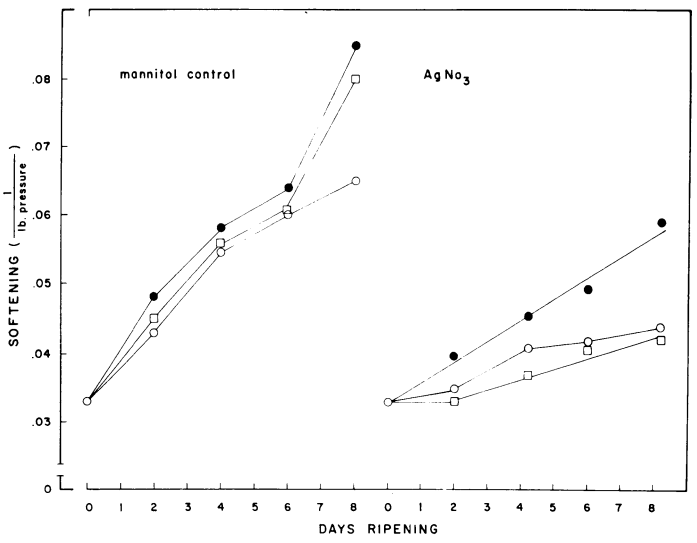

Fig. 5. The softening of pears infiltrated with carrier mannitol solution (0.3 M) and mannitol plus 10^{-4} M silver nitrate as occurring in air (○), and as effected by 10 μl/liter ethylene (□), or 1,300 μl/liter acetaldehyde (●).

fruit ripening (8).

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