Table 3. Comparison of total residues (acephate plus methamidophos) on greenhouse tomatoes from either a single or multiple application.

Rate of application (kg/ha)	Type of application	Total residues (ppm) Day 2	Day 3	Day 4
0.56	Single	0.22	0.16	0.08
	Multiple[z]	0.43	0.37	2.25
1.12	Single	1.14	0.30	0.27
	Multiple[z]	1.86	0.78	0.47
2.24	Single	0.75	0.34	0.09
	Multiple[z]	3.03	1.96	0.85

[z]Plots sprayed once a week for 4 weeks and harvested 2, 3, and 4 days after the last application.

greater after a multiple-spray application than after a single application (Table 3). Total residues in ppm from multiple applications were from 2 to 9 times greater than those from a single application

Discussion

The observed variation of residue levels of acephate and methamidophos could be due to several factors, but tomato size was probably of major importance. The concn of residue in a large fruit with a lower surface area to weight ratio would be smaller than that for a small fruit with a larger surface area to weight ratio with the same deposit of spray per unit area on the fruit surface. The generally lower acephate residues in expt. 2 (after single application) might be due, also, to the spring vs. fall planting and differences in the environmental conditions in the greenhouse.

Fruit varied from 4 to 12 cm in diam in most samples; and differences in fruit size probably contributed to variations of the residue values. Fruit for all 3 harvests following the 4 weekly sprays were smaller (ranging from 4 to 7 cm in diam with some immature fruit) than those from the harvests after the single application. Therefore, differences between residues on samples harvested after 4 weekly sprays and those on samples after the single spray can be attributed, at least in part, to the effect of the surface area to weight ration on residues. Some of the difference might also have been due to accumulation of residues on samples that received multiple sprays.

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J. Amer. Soc. Hort. Sci. 103(3):394–397. 1978.

Inhibition of Ripening Processes in Pears by Inhibitors of Cyanide-resistant Respiration and by Silver[1]

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Additional index words. cyanide-insensitive respiration, *Pyrus communis*

Abstract. **Various concentrations of salicylhydroxamic acid and alpha, alpha-dipyridyl (reported inhibitors of cyanide-insensitive respiration) applied to fruit of 'Bosc' pear (*Pyrus communis* L.) reduced fruit softening. The application of silver ions, reported to inhibit ethylene action, delayed ripening.**

The initiation of the ripening process in climacteric fruit is attributed to the action of ethylene as it increases in tissues approaching senescence (5). The action of ethylene in promoting fruit ripening can be minimized by rapidly removing this volatile from fruit tissues. This is done by increasing the diffusion of ethylene to the environment by storing fruit under hypobaric conditions (6). The action of ethylene can also be antagonized by high CO_2 and low oxygen tensions (7). Recently Beyer (3) showed that silver will antagonize such ethylene-dependent responses in plants as leaf, flower, and fruit abscission in cotton, floral senescence in orchids, and the "triple response" in etiolated peas. Other methods to antagonize the action of ethylene may be based on inhibiting the metabolic action of the compound. Solomos and Laties (16) suggested that ethylene stimulates the cyanide-resistant respiration (the "alternate respiratory pathway"). In this view, stimulating fruit ripening by ethylene may reflect the initiation of the cyanide-resistant respiration (15). Consequently, inhibiting this respiratory path may result in a corresponding inhibition of ethylene-stimulated ripening processes in fruit.

In the present study, we examined this concept and we show that inhibitors of the cyanide-resistant respiration inhibit ripening processes in pear. Likewise, silver, the ethylene antagonist, is a potent inhibitor of fruit ripening.

Materials and Methods

'Bosc' pear, obtained from Pine Grove, Pa, were harvested in the mature-green preclimacteric state and stored under hypobaric conditions at 0°C until used (up to 2 months). Average fruit wt was 125 g. Hypobaric conditions were 1/10th atmospheric pressure with the continuous flow of water saturated air through the chamber to prevent disication.

A vacuum infiltration technique (10) was employed to apply test compounds to intact fruit. The test solutions were applied in a 0.3 M mannitol solution as a carrier. The fruits were infiltrated at an average rate of 5 ml of test solution/100 g of

[1]Received for publication Nobember 18, 1977.

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fruit tissue. Control fruits were infiltrated with the mannitol solution only. The test compounds represented inhibitors of the cyanide-resistant respiration, including salicylhydroxamic acid (SHAM) (employed at concn of 10^{-3} M and 10^{-2} M) and alpha, alpha-dipyridyl (Sigma Chemical Co., St. Louis, Mo.) (employed at concn of 10^{-3} M, 3×10^{-3} M, and 10^{-2} M) or the ethylene antagonist, silver nitrate (Fisher Scientific Co., Springfield, N.J.), (employed at concn of 10^{-4} M, 10^{-3} M, and 10^{-2} M).

After infiltration, the fruit was placed in glass jars and ventilated with ethylene-free air at a rate of 400 ml/min. Ethylene was scrubbed from the ventilating air with Purafil (H. E. Burrough and Assoc., Chamblee, Ga.). The experiments were conducted at room temperature (22° ± 2°C). Ten fruit were used for the daily measurement of CO_2 and theylene evolution, and on alternate days, for softening. Respiration was measured using a Beckman infra-red CO_2 analyzer. Ethylene was collected from the ventilating gases, according to the method of Young et al (21). Fruit softening was measured using a Magness-Taylor fruit pressure meter, as previously described with 10 fruit being tested during each reading (9). Samples of 10 fruit were used for each of the above determinations. Each experiment was repeated at least twice.

Results and Discussion

The effect of SHAM and alpha, alpha-dipyridyl on the onset of the climacteric, ethylene evolution, and softening, is shown in Fig. 1–3. Both compounds were used as inhibitors of the cyanide-resistant respiration, and were used in the present experiment to test their effect on the respiratory upsurge in ripening pears. SHAM did not inhibit the climacteric respiration (Fig. 1). On the contrary, the CO_2 output increased progressively as the concn of the applied compound was increased. By comparison, alpha, alpha-dipyridyl inhibited fruit respiration at the highest concn of 10^{-2} M.

The effect of these compounds on ethylene synthesis, was also different (Fig. 2). SHAM showed some inhibition in proportion to the employed concn, whereas alpha, alpha-dipyridyl stimulated ethylene synthesis at a lower concn (10^{-3} M or 3×10^{-3} M), but was strongly inhibitory at 10^{-2} M.

Although these compounds may have retarded the cyanide-resistant respiration in fruit, as was shown in other systems (2, 14), the present results suggest that the respiratory upsurge in ripening fruit may not reflect the cyanide-resistant path

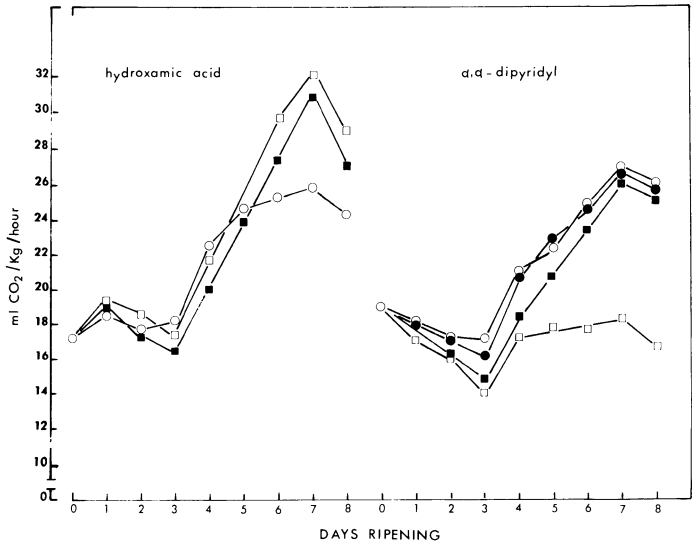

Fig. 1. The effects of various concn of salicylhydroxamic acid (zero (○), 10^{-3} M (■), 10^{-2} M (□)), and alpha, alpha-dipyridyl (zero (○), 10^{-3} M (●), 3×10^{-3} M (■), 10^{-2} M (□)) on respiration, measured as CO_2 production.

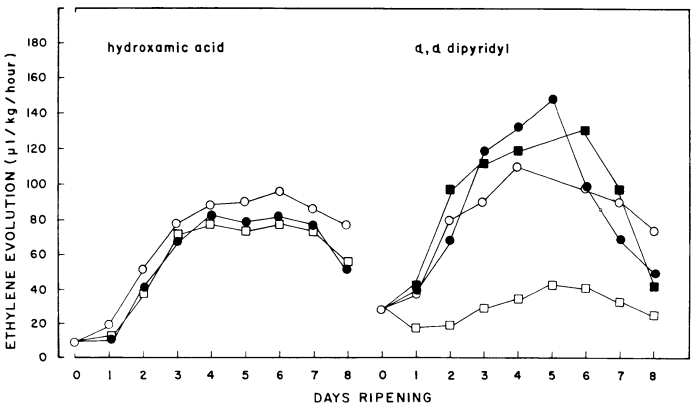

Fig. 2. The effects of the inhibitors salicylhydroxamic acid and alpha, alpha-dipyridyl on ethylene production in pears. The concn used were: for salicylhydroxamic acid (zero (○), 10^{-3} M (●), 10^{-2} M (□)) and for alpha, alpha-dipyridyl (zero (○), 10^{-3} M (●), 3×10^{-3} M (■), 10^{-2} M (□)).

exclusively. The hydroxamic acid actually promoted respiration, while alpha, alpha-dipyridyl inhibited greatly only at the highest concentration.

The results also show an inverse relationship between CO_2 and ethylene evolution as influenced by the test compounds. While the salicylhydroxamic acid promoted CO_2 evolution, it slightly inhibited ethylene synthesis. Likewise, alpha-alpha-dipyridyl induced a slight inhibition of CO_2 evolution at the lower concn (10^{-3} M and 3×10^{-3} M), but promoted ethylene synthesis. Only at the highest concn (10^{-2} M) did this compound effectively inhibit the evolution of both CO_2 and ethylene. These results suggest that, although the evolution of CO_2 and ethylene that occur normally in ripening fruit appear to be synchronized, each may have different metabolic origins which could be influenced differently. These results are supported by those of Wang and coworkers (18, 19, 20) who have shown that in pear the ethylene production, and climacteric rise can procede independently as the fruit ripens. Since both processes were similarly inhibited by high concn of alpha, alpha-dipyridyl, this may reflect a more widespread effect, and may indicate, as suggested by Tetley and Thimann (17), a partial inhibition of protein synthesis on which fruit ripening depends (9).

Both test compounds were equally effective in inhibiting softening, showing a progressively greater effect as their concn

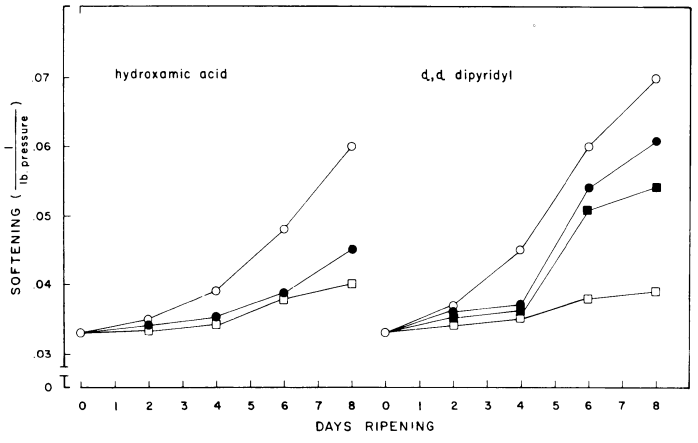

Fig. 3. The influence of salicylhydroxamic acid (zero (○), 10^{-3} M (●), 10^{-2} M (□)) and alpha, alpha-dipyridyl (zero (○), 10^{-3} M (●), 3×10^{-3} M (■), 10^{-2} M (□)) on softening.

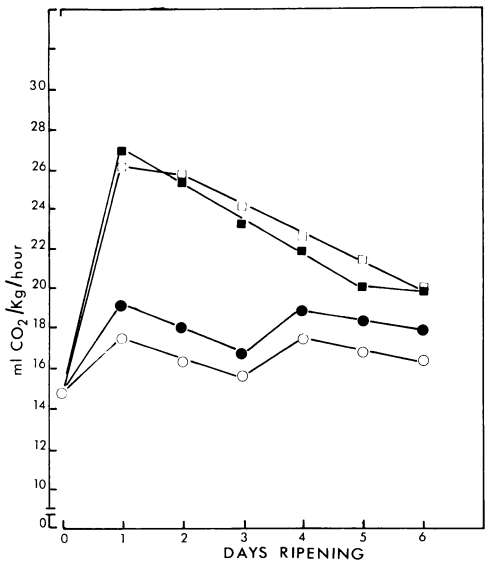

Fig. 4. Respiration of pears as effected by various concn of silver nitrate (zero (○), 10^{-4} M (●), 10^{-3} M (■), 10^{-2} M (□)).

increased (Fig. 3). At the highest concn (10^{-2} M) inhibition was almost complete. These results show that, while CO_2 and ethylene evolution in ripening fruit may not be directly related to the cyanide-resistant respiration, the softening process may reflect dependence on the activity of this pathway. Previously it was shown that the activity of the cyanide-resistant pathway led to the formation of H_2O_2 in isolated plant mitochondria (12) and intact potato tubers (8) and, thereby, possibly contributed to the rise in peroxides that accompanies the ripening process in pears (4). The action of peroxides has been related to the cell wall softening that occurs in aging fruit tissues (4) or to the action of pathogens (11). According to this view, the inhibition of peroxide formation resulting from the action of the cyanide-resistant path, may limit cell wall degradation and thereby softening. Other mechanism(s) of ethylene action are possible, however, and a direct effect of the compounds on cell wall metabolism cannot be excluded, particularly when we notice that SHAM can stimulate CO_2 production

The effect of silver nitrate on respiration, ethylene evolution

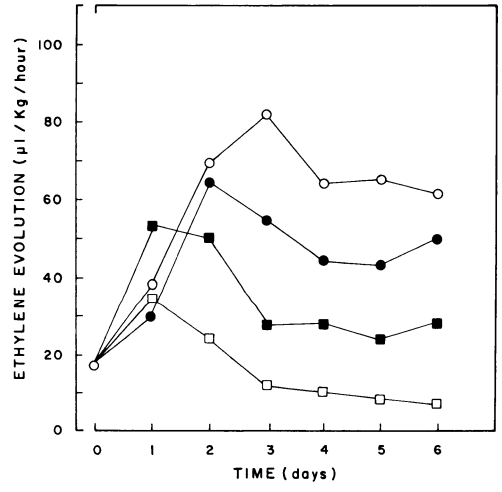

Fig. 5. The inhibition of ethylene synthesis by silver nitrate (zero (○), 10^{-4} M (●), 10^{-3} M (■), 10^{-2} M (□)).

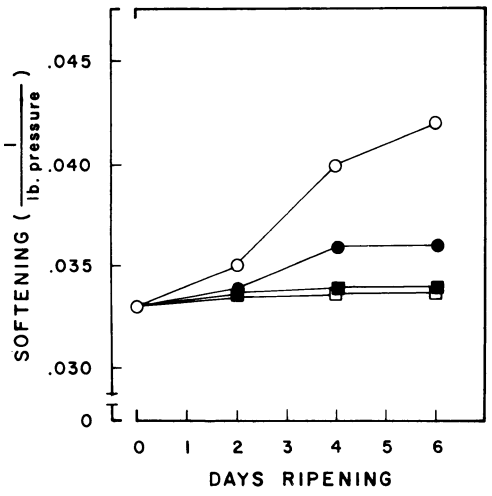

Fig. 6. The effect of silver nitrate on softening in pears. The concn used were: (zero (○), 10^{-4} M (●), 10^{-3} M (■), and 10^{-2} M (□)).

and softening, is shown in Fig. 4–6. As with SHAM, the increase in the concn of the compound led to a progressive stimulation in respiration (Fig. 4) a decline in ethylene evolution (Fig. 5), and a marked inhibition of fruit softening (Fig. 6). Beyer (3) showed that silver antagonized the action of ethylene in cotton leaf abscission and induction of senescence in orchids. The present results extend these observations to fruit ripening, showing that silver is a potent inhibitor of cell wall softening. Likewise, the application of silver led to a decline in the production of ethylene. Beyer showed that in other systems the application of silver was not associated with reduced ethylene production, although out data show inhibited ethylene production. In pear, as in other climacteric fruit, the synthesis of ethylene is autocatalytic (1) and, therefore, blocking the action of ethylene may cause diminution in the synthesis of the compound.

The present results and other data (9, 13) show that the onset of characteristic ripening processes, such as softening, and the accompanying respiratory upsurge can be regulated differentially as can be observed when the fruit is treated with SHAM or silver nitrate. It has also been shown by Wang and his coworkers (18, 19, 20) that 3 of the ripening parameters including ethylene production, softening, and climacteric are separate reactions proceeding independently during the ripening process. The relationship of the respiratory climacteric to ripening may, therefore, be indirect. By comparison the treatments that inhibited the synthesis or the action of ethylene also led to the inhibition of softening, suggesting a closer interdependency between the onset of ripening and ethylene metabolism. Although, ethylene appears to be directly involved in regulating ripening, the action of the compound may not rely exclusively on the stimulation of the alternate respiratory path. Other ethylene sensitive pathways may be involved in stimulating ripening.

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J. Amer. Soc. Hort. Sci. 103(3):397–400. 1978.

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

[1]Received for publication November 18, 1977.
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