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Effect of Vitamin K_5 and Menadione on Ripening, and Ethylene and Carbon Dioxide Production by Apple and Tomato Fruit[1]

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Abstract. **A 10-minute soak in 1.0 mM vitamin K_5 reduced ethylene production over 90%, while doubling carbon dioxide production by cortical tissue from pre-climacteric apples (*Malus domestica* Borkh.). Reduced ethylene production persisted for at least 4 hours, while carbon dioxide production declined to rates not significantly different from the controls. Vitamin K_5 also reduced ethylene production by 50% from quartered fruit of tomato (*Lycopersicon esculentum* Mill.) at different stages of maturity, and from cortex tissue from apples at or near their climacteric peak of ethylene production.**

Vitamin K_1 (2-methyl-3-phytyl-1,4-naphthoquinone) and menadione (2-methyl-1,4-naphthoquinone) have been reported to retard the ripening of banana fruit (3) and tomato fruit (M. B. Farhcomand and M. E. Patterson, personal communication). Farhcomand and Patterson observed that soaking mature-green tomatoes for 10 min in either a 0.1% (wt/vol) emulsion of oil soluble menadione, or a 0.4% solution of water soluble menadione sodium bisulfite (MSB) delayed ripening for 35 days when the tomatoes were held at 20°C. These treatments also significantly reduced ethylene and carbon dioxide production. Beccari (3) reported that soaking mature-green bananas for 5 min in either a 0.1% emulsion of vitamin K_1, or a 0.1% solution of a water soluble form of menadione delayed ripening of fruit held at 20 and 30°C for 33 and 24 days, respectively. In contrast, Peacock (7) found that a 0.1% solution of menadione actually promoted banana ripening when precautions were taken to control fungal infection. He suggested that the results reported by Beccari were caused by reduction of anthracnose infection by menadione. Beccari was aware that a 0.1% solution of either vitamin K_1, or menadione would markedly reduce the growth of *Gloeosporium musarum* Cke Massee, and *Fusarium* sp. prope *monififorme* Sh. in culture, but he did not attribute the delay in ripening to this anti-fungal property. A 2-min soak in a 0.1% menadione solution has been reported to be much more effective than 8 other anti-fungal compounds in inhibiting the growth of *Rhizoctonia bataticola,* the fungus responsible for blackspot on mango (4). More effective than menadione is the synthetic compound vitamin K_5 (4-amino-2-methyl-1-naphthol), which has been shown to greatly inhibit microbial growth at concentrations of less than 50% ppm (0.24 mM) (10).

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Several naturally occurring and synthetic forms of vitamin K are known (6, 10). Vitamin K_1, first isolated from alfalfa, is widespread in higher plants (6), and functions in the electron-transport chain (5, 9). Menadione is a synthetic compound which animals can convert to vitamin K_2 by adding a phytyl side-chain of from 1 to 13 isoprenoid units at the 3 position. Menadione is insoluble in water, but 1 g of MSB is soluble in about 2 ml of water. Vitamin K_1, vitamin K_5, menadione, and MSB decompose at different rates in air, but all are readily decomposed by light or alkaline solutions. Menadione is sometimes improperly referred to as vitamin K_3 (6).

Menadione and vitamin K_1 would be useful in postharvest handling of perishable fruit if they retard ripening as reported. This paper reports the effects of menadione, MSB, and vitamin K_5 on ripening, and on ethylene and carbon dioxide production by apple and tomato fruit at various stages of ripening.

Materials and Methods

Fruit tissue. Mature unripe 'Starkrimson Delicious' and 'Golden Delicious' apples were obtained from research plots. Cylinders (2 x 1 cm diam) and disks (2 mm x 1 cm diam) were cut from the cortex of fruit which were freshly harvested, or had been stored at -0.5^{o}C. All fruit were warmed to 23^{o} for at least 2 hr before being used in an experiment.

Fruit of 'Tiny Tim' tomato were obtained from greenhouse-grown plants. Experiments either used uniform fruit ranging from mature-green to red-ripe, or a heterogenous aged population of freshly harvested mature-green fruit which varied in weight from 4 to 12 g per fruit.

After sorting into uniform experimental groups, the whole or quartered tomatoes, and the whole apples or excised cortex tissue were subjected to the various treatments. All experiments were repeated at least 3 times with from 20 to 100 replicates in the whole fruit soaking and vacuum infiltration experiments, and with at least 3 replicates when cortex tissue or quartered tomato sections were used. Each fruit was used as a block, with cylinders, disks, or sections subjected to all treatments.

Solutions. Depending on the fruit tissue being used, various aqueous solutions, or emulsions of vitamin K_5, menadione, and menadione sodium bisulfite (MSB) were made by sonicating the compounds in one of the following solutions for 15 min. Whole fruit were either soaked in solutions containing distilled water and 0.1% (by vol) Tween 20, or vacuum infiltrated with solutions containing 0.5 M glycerol and 5.0 mM $CaCl_2$. Quartered tomatoes were soaked in solutions containing 0.5 M glycerol. Cortex cylinders and disks were either soaked in, or vacuum infiltrated with solutions containing 0.5 M glycerol and 5.0 mM $CaCl_2$. The pH of some solutions were adjusted by using 50 mM citric acid buffer (for pH 3 to 5) or HEPES buffer (for pH 6 to 9).

Treatments. Whole or quartered tomato fruit were either soaked in the appropriate solution for 10 min, or vacuum infiltrated with the solution in the following manner. The fruit were placed in a large beaker or vacuum desiccator, and enough solution was added to cover the fruit which were kept submerged with a weighted cover. A vacuum of 150 mm Hg was maintained for 30 min. After the treatments, the fruit were transferred to trays covered with cloth towels and kept at 23^{o}C in room light, or in the dark. Ethylene and carbon dioxide production was periodically measured by taking 1-ml gas samples from the headspace of containers which had enclosed individual fruit for 1 hr, and analyzed as previously described (8). Whole fruit showing any red color development were considered to be ripening, and were separated from the other fruit. These ripening fruit were kept for observation and all turned fully red in 2 to 3 days. Another measure of ripening was taken as the rise in ethylene production. Daily records of ethylene production were kept for each fruit. The beginning of the ethylene climacteric was considered to occur on the day ethylene production increased over 0.3 nl g^{-1} hr^{-1}, if the rate of production continued to increase for 3 days.

Freshly harvested apples were soaked once, or every 4 days for 15 min. In other experiments, cortex cylinders and disks were excised with a cork borer, trimmed to the required length, soaked for 15 min in the appropriate solution, blotted dry, and enclosed in 10-ml glass syringes for 1 hr. One-ml gas samples were taken and analyzed for ethylene and carbon dioxide as previously described (8). For repeated measurements, the syringes were flushed for 5 min with humidified ethylene-free air between 1-hr accumulation periods.

Results and Discussion

Tomatoes. Soaking or vacuum infiltrating whole tomato fruit with various concentrations of menadione and MSB did not delay ripening as measured by red color development (Fig. 1), or reduce ethylene or carbon dioxide production (data not shown). Similar results were obtained when experiments were performed in the light or in the dark. Since the epidermis and cuticle of whole fruit may have retarded solution penetration into the tissue, quartered tomatoes were soaked in similar solutions to insure tissue exposure. As with whole fruit, menadione and MSB were ineffective in reducing ethylene or carbon dioxide production (data not shown). However, a 10-min soak in vitamin K_5 significantly reduced ethylene production by quartered tomatoes at 3 stages of ripeness (Table 1). A 1.0 mM concentration of vitamin K_5 reduced ethylene production by 58% from quartered mature-green fruit, by 52% from orange fruit, and by 65% from red-ripe fruit. At 10 mM, vitamin K_5 continued to inhibit ethylene production from quartered mature-green fruit (by 77%), and orange fruit (by 73%), but stimulated ethylene production by red-ripe fruit.

To see if ripening would also be inhibited by vitamin K_5, groups of 16 mature-green fruit were vacuum infiltrated with 0.0 to 10 mM vitamin K_5 solutions. Only the 10 mM vitamin K_5 solution was effective. The rate of ethylene production increased the day after treatment for both the 10 mM vitamin K_5 treated and control fruit (Fig. 2). It continued to slowly

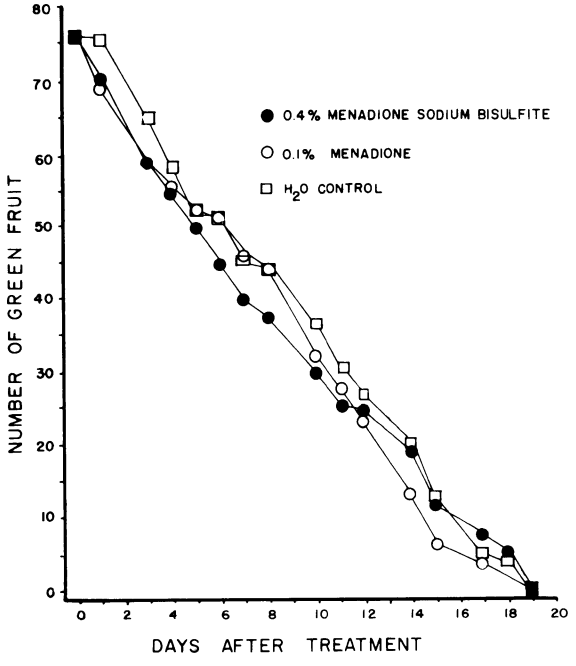

Fig. 1. Effect of a 20-min soak in 0.1% (v/v) Tween 20 (H_2O control), plus or minus 0.1% (wt/vol) menadione or 0.4% menadione sodium bisulfite on the development of red color in a heterogenous population of 'Tiny Tim' tomatoes.

Table 1. Effect of a 10-min soak in various concentrations of vitamin K_5 on the rate of ethylene production by quartered 'Tiny Tim' tomatoes at 3 stages of ripeness.

Vitamin K_5 (mM)	nl C_2H_4/g·hr		
	Stage of ripeness		
	Green	Orange	Red
0.0	1.38	11.55	2.81
0.1	1.19	10.01	1.94
1.0	0.58	5.58	0.98
10.0	0.32	3.56	1.35
LSD 5%	0.50	3.67	0.87

increase in the control group during the experiment, but immediately declined to pre-treatment levels in the vitamin K_5 treated group. This low level of ethylene production by the treated fruit did not significantly change until the 10th day after treatment. The average rate of ethylene production from the treated tomatoes then rapidly increased and surpassed the control group on day 13. Infiltration with 10 mM vitamin K_5 solutions also reduced the number of fruit starting their ethylene climacteric (Fig. 3). In contrast, the control fruit showed a steady increase in the number of climacteric fruit. However, 10 days after treatment, the vitamin K_5 treated group had as many climacteric fruit as the control group. The number of climacteric fruit sharply increased after 10 days; surpassing the control group 2 days later.

The 10-fold higher concentration of vitamin K_5 necessary to elicit an inhibitory response in whole fruit, as compared to quartered fruit, may have resulted because the stem scar provided an efficient filter which removed the finely derived flocculent suspension of vitamin K_5 from the infiltrating solution. Although research had proceeded concomitantly with tomatoes and apples, the difficulty in obtaining suitable tomato fruit, the heterogenity of tomato tissue, and the difficulty of getting good solution penetration, prompted the decision to use apple cortex tissue in subsequent experiments.

Apples. Soaking whole apples in various concentrations of menadione and MSB did not significantly affect ethylene or carbon dioxide production (data not shown). Subsequent experiments were performed on excised cortex tissue because of its uniformity and the ease of solution penetration. Excision

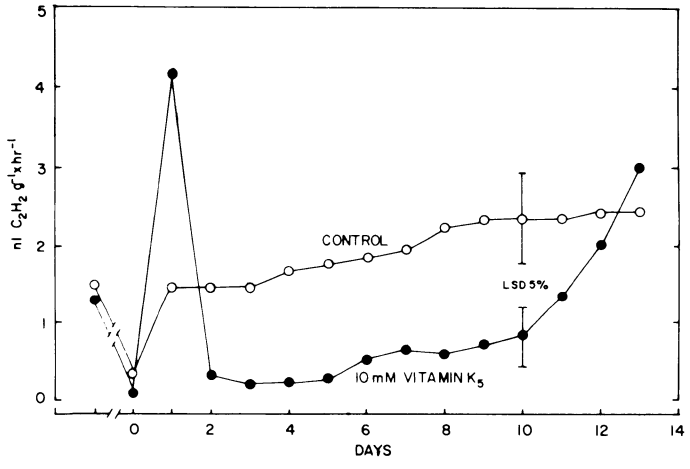

Fig. 2. Effect of vacuum infiltrating 'Tiny Tim' tomatoes with 0.5 M glycerol, 5.0 mM $CaCl_2$, 50 mM citric acid buffer plus or minus 10 mM vitamin K_5 (solution adjusted to pH 3.5) on ethylene production.

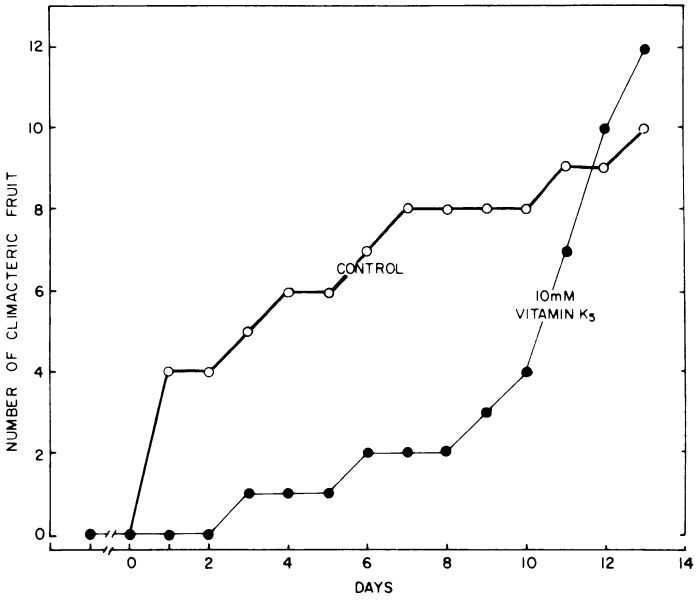

Fig. 3. Effect of vacuum infiltrating 'Tiny Tim' tomatoes with 0.5 M glycerol, 5.0 mM $CaCl_2$, 50 mM citric acid buffer plus or minus 10 mM vitamin K_5 (solution adjusted to pH 3.5) on the time to the beginning of the ethylene climacteric for individual fruit.

of 2 x 1 cm diam cortex cylinders did not significantly increase ethylene production for up to 4 hr, from 29.9 nl g^{-1} hr^{-1} for whole fruit to 31.3 nl g^{-1} hr^{-1} for cylinders. However, carbon dioxide production was stimulated 49% from 15.2 μl g^{-1} hr^{-1} for whole fruit to 22.6 μl g^{-1} hr^{-1} for excised cylinders. Soaking cortex cylinders for 10 min in various concentrations of menadione (data not shown) or MSB did not significantly affect ethylene production (Table 2). At the higher concentrations of MSB, carbon dioxide production was stimulated over the controls after 4 hr. Similar results were obtained with cylinders taken from pre-climacteric apples, apples starting their climacteric rise, and climacteric fruit.

Soaking cortex cylinders from 'Starkrimson Delicious' and 'Golden Delicious' apples for 10 min in various concentrations of vitamin K_5 significantly reduced ethylene production, while stimulating carbon dioxide production (Table 3). As the concentration of vitamin K_5 was increased, ethylene production was first slightly stimulated, before being reduced at concentrations above 0.3 mM. This pattern was still evident after 4 hr.

Table 2. Effect of a 10-min soak in various concentrations of menadione sodium bisulfite (MSB) on the rate of ethylene and carbon dioxide production by cylinders of cortex tissue from 'Golden Delicious' apples starting their climacteric rise.

MSB (mM)	nl C_2H_4/g·hr		μl CO_2/g·hr	
	0-1 hr	4-5 hr	0-1 hr	4-5 hr
0.00	63.8	48.1	28.4	10.0
0.01	63.0	48.4	28.8	10.4
0.03	51.6	37.3	29.1	11.4
0.10	56.6	44.9	29.4	11.5
0.30	57.7	41.9	28.8	11.6
1.0	60.1	48.0	28.5	11.9
3.0	55.7	47.4	26.7	12.6
10.0	51.6	47.3	26.5	15.0
LSD 5%	NS	NS	NS	2.6

Table 3. Effect of a 10-min soak in various concentrations of vitamin K_5 on the rate of ethylene and carbon dioxide production by cortex cylinders of 'Starkrimson' and 'Golden Delicious' apples starting their climacteric rise.

Vitamin K_5 (mM)	nl C_2H_4/g·hr				μl CO_2/g·hr			
	Starkrimson		Golden Delicious		Starkrimson		Golden Delicious	
	0-1 hr	4-5 hr	0-1 hr	4-5 hr	0-1 hr	4-5 hr	0-1 hr	4-5 hr
0.00	7.4	11.7	62.0	76.9	23.7	15.8	32.5	18.7
0.01	7.4	13.8	74.8	82.3	28.4	16.4	38.4	20.2
0.03	6.1	10.3	76.2	87.0	28.3	16.1	39.0	20.4
0.10	11.8	14.4	73.4	80.7	37.3	17.4	45.1	19.1
0.30	11.0	13.0	53.7	71.6	49.1	14.5	58.2	18.1
1.0	0.37	6.4	17.7	56.0	59.7	17.8	65.8	16.8
3.0	0.11	5.3	0.03	25.8	57.9	12.2	68.1	18.1
10.0	0.06	6.9	0.04	42.5	50.2	5.7	64.6	13.5
LSD 5%	4.6	2.9	16.8	26.1	20.2	4.1	7.8	3.1

Table 4. Effect of a 10-min soak in various concentrations of vitamin K_5 on the rate of ethylene and carbon dioxide production by cylinders of cortex tissue from climacteric 'Golden Delicious' apples.

Vitamin K_5 (mM)	nl C_2H_4/g·hr	μl CO_2/g·hr
0.00	267	54
0.01	218	57
0.1	244	60
1.0	191	90
10.0	133	84
LSD 5%	49.7	11.2

Ethylene production by tissue from 'Golden Delicious' apples was also reduced at concentrations of vitamin K_5 above 0.3 mM, but the reduction was less dramatic and less persistent than with tissue from 'Starkrimson Delicious' apples. This difference may be because the 'Golden Delicious' fruit were further into their ethylene climacteric; producing almost 10-fold as much ethylene as the 'Starkrimson Delicious' fruit. As with tomatoes (Table 1), vitamin K_5 was less effective in reducing ethylene production by more mature tissue (Table 4). Soaking cortex cylinders from 'Golden Delicious' apples in 1.0 mM vitamin K_5 reduced ethylene production by 95% for pre-climacteric tissue, by 71% for tissue starting their climacteric rise, and by 28% for climacteric tissue.

In contrast to its effect on ethylene production, concentrations of vitamin K_5 which first reduced ethylene production, actually stimulated carbon dioxide production by the same tissue (Table 3). After 4 hr, carbon dioxide production continued to be stimulated by 1.0 mM vitamin K_5, but had recovered to control rates, or below, for all other concentrations.

Between pH 3 and 9, the solution pH had no significant effect on the ability of a 5.0 mM vitamin K_5 solution to reduce ethylene production, but solutions with a high pH significantly stimulated carbon dioxide production (Table 5). A pH of 3.5 was selected for subsequent experiments because it had little effect on carbon dioxide production, and because the vitamin K_5 solutions appeared to be more stable at this pH.

Cutting 2 × 1 cm diam cortex cylinders into 2 mm × 1 cm diam disks did not significantly affect the rate of ethylene production. However, it greatly increased the effectiveness of a 10-min soak in 2.0 mM vitamin K_5 in reducing ethylene production by almost 3-fold; from a 36% reduction with whole cylinders to a 96% reduction in ethylene production from disks. Maximum inhibition of ethylene production after a 1-min soak in 5 mM vitamin K_5 solution occurred 30 min after the treatment of disks, and 90 min after the treatment of cylinders. The response was faster and the degree of inhibition greater with disks. Since cutting the cylinders into smaller portions, and finally into disks, increased the surface area to about the same degree as it increased the effectiveness of vitamin K_5 (e.g. a 2.4-fold increased surface area versus a 2.6-fold increased vitamin K_5 activity for disks), it seems reasonable to assume that the increased effectiveness of vitamin K_5 was simply due to increased tissue exposure to the solution.

Concentrations of vitamin K_5 up to 0.1 mM had no significant effect on the rate of ethylene or carbon dioxide production from apple cortex disks soaked for 10 min (Fig. 4, 5). However,

Table 5. Effect of a 10-min soak in solutions containing 0.5 M glycerol, 5 mM $CaCl_2$, and 50 mM buffer plus or minus 5 mM Vitamin K_5, and adjusted to various pH values, on the rate of ethylene and carbon dioxide production by cortex cylinders from 'Golden Delicious' apples.

pH	nl C_2H_4/g·hr			μl CO_2/g·hr		
	Control	Vitamin K_5	Reduction (%)	Control	Vitamin K_5	Increase (%)
3	99.5	59.5	40.2	47.6	64.2	34.9
4	93.0	60.6	34.8	49.1	61.0	24.2
5	91.2	57.6	36.8	49.2	58.3	18.5
6	84.6	57.4	32.2	39.6	62.4	57.6
7	86.1	57.6	33.1	33.5	56.7	69.3
8	105.3	60.1	42.9	27.2	50.6	86.0
9	89.7	47.0	47.6	24.0	44.7	86.3
LSD 5%	NS	NS		4.9	7.2	

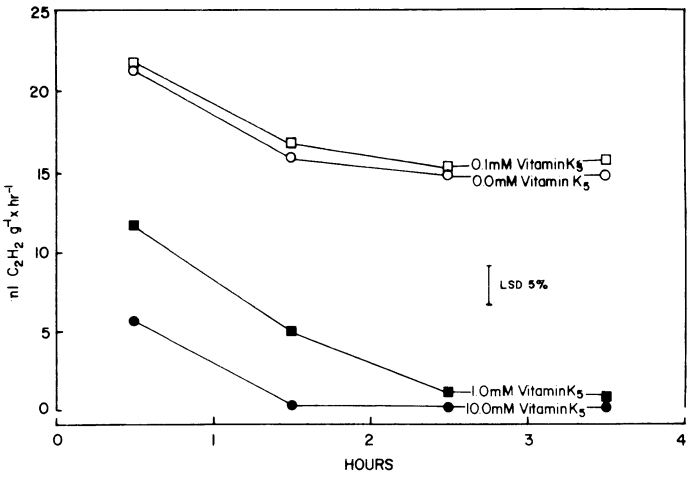

Fig. 4. Effect of a 10-min soak in 0.5 M glycerol, 5.0 mM $CaCl_2$, 50 mM citric acid buffer with 0, 0.1, 1.0, and 10 mM vitamin K_5 (solutions adjusted to pH 3.5) on ethylene production by 2 mm x 1 cm diam cortex disks from 'Golden Delicious' apples.

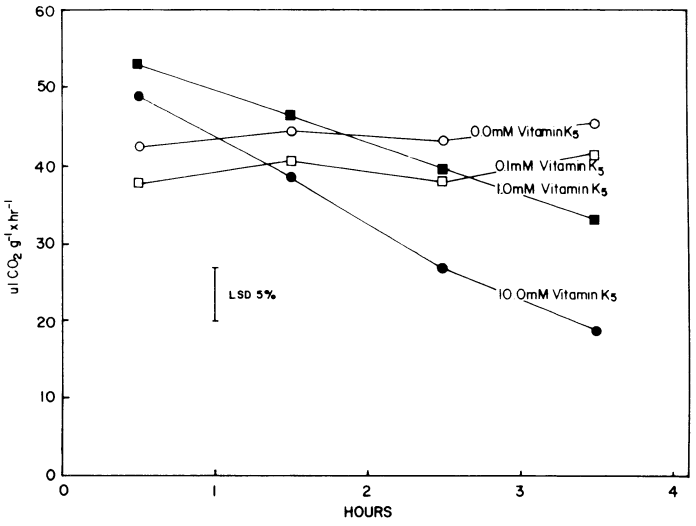

Fig. 5. Effect of a 10-min soak in 0.5 M glycerol, 5.0 mM $CaCl_2$, 50 mM citric acid buffer with 0, 0.1, 1.0, and 10 mM vitamin K_5 (solutions adjusted to pH 3.5) on carbon dioxide production by 2 mm x 1 cm diam cortex disks from 'Golden Delicious' apples.

above 0.1 mM vitamin K_5, ethylene production was progressively inhibited with time and concentration (Fig. 4), while carbon dioxide production was first stimulated and then inhibited (Fig. 5). Unlike treated cylinders (Table 3) in which ethylene production started to recover after 4 hr, ethylene production by disks was almost completely inhibited by 1.0 to 10.0 mM vitamin K_5 within 4 hr. The tissue did not recover detectable ethylene production even after 12 hr (data not shown). Carbon dioxide production continued to drop for 5 hr; stabilizing at around 50% of the control rate after 6 hr for both the 1.0 and 10.0 mM vitamin K_5 treated disks.

ACC (1-aminocyclopropane-1-carboxylic acid) has recently been shown to be the last stable intermediate in the biological conversion of methionine to ethylene (1). Ethylene production by apple cortex disks was stimulated from around 20 nl g^{-1} hr^{-1} to over 220 nl g^{-1} hr^{-1} during the first hr exposure to a 0.5 mM ACC solution. After 4 hr, the control tissue was continuing to produce ethylene at around 20 nl g^{-1} hr^{-1}, while the ACC treated tissue had increased production to around 400 nl g^{-1} hr^{-1}. Making the ACC solutions 1.0 mM in vitamin K_5 after 2 hr of treatment, caused ethylene production to be reduced to near zero rates within 2 hr. Timing of the vitamin K_5 inhibition of ethylene production was the same for control and ACC treated tissue. Free radical quenchers such as propyl gallate and catechol also reduced normal and ACC stimulated ethylene production, but these compounds were less effective than vitamin K_5.

On a molar basis, vitamin K_5 appears to be almost as potent an inhibitor of ethylene production from apple and tomato tissue as are silver ions (8). However, unlike the silver ion which is highly toxic, vitamin K_5 is safe enough to be used as a food preservative (10). A major deterrent to commercial use of vitamin K_5 would be its decomposition in air and light. This deficiency could possibly be remedied by using a vitamin K_5 analog which retains the ability to reduce ethylene production and is chemically stable. There are many water-soluble analogs which possess vitamin K activity (2).

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