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Silver Ion Inhibits Ethylene Synthesis and Action in Ripening Fruits[1]

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Abstract. **Ag(I), applied in aqueous solution as $AgNO_3$, inhibited ethylene synthesis and ripening of mature green banana fruit slices and pericarp discs of mature green tomato fruit. It also inhibited ethylene production by cortical tissue from post-climacteric apples. Concentrations of Ag(I) which reduced ethylene synthesis had an inconsistent effect on CO_2 production: no effect on banana slices (1.0 mM Ag^+), stimulation in apple tissue (0.1 mM Ag^+), and inhibition in tomato tissue (0.3 mM Ag^+). This was accompanied by a slight amount of tissue necrosis at these concentrations. Of the 7 metallic salts tested, only Ag(I) inhibited ripening and ethylene synthesis at observed non-phytotoxic levels. Inhibition of ripening and ethylene synthesis by Ag(I) was evident in tissue treated with sufficient exogenous ethylene to elicit both responses in control tissue. The inability of applied ethylene to overcome the inhibitory effect of Ag(I) suggests that the silver ion may interfere with the primary action of ethylene in the tissue.**

Ethylene, whether exogenously applied or endogenously produced, promotes the ripening of mature fruits and vegetables (5, 10, 13). Reducing the endogenous concn of ethylene by hypobaric ventilation (3), with inhibitors of ethylene synthesis (10), or by reducing the activity of ethylene with elevated concn of CO_2 (4) delays ripening. Beyer (2) recently showed that the silver ion, Ag(I), applied foliarly as a solution of $AgNO_3$, was far more effective than 9 other metal ions at inhibiting the effect of exogenous ethylene on etiolated pea stems, cotton plants, and orchid flowers. A short dip in a 50 ppm $AgNO_3$ solution extended the longevity of carnation flowers by reducing their sensitivity to ethylene (8). In contrast, impregnating the stem with 1000 ppm silver solutions extended flower life by inhibiting the growth of microorganisms (12). Lau and Yang (9) have reported that ethylene production by post-climacteric apple tissue and by etiolated mung bean hypocotyl tissue was inhibited by Co^{++}, and to a lesser extent by Ni^{++}.

In this paper we report the inhibitory effect of Ag(I) on ethylene synthesis by apple tissue and on ethylene synthesis and ripening of banana and tomato fruit tissue.

Materials and Methods

Preparation of tissue. Cylinders (3 x 1 cm diam) cut from the cortex of post-climacteric apples (*Malus domestica* Borkh. cv. Idared) were soaked for 30 min in 3 changes of 0.5 M glycerol, blotted dry, weighed, and placed in test tubes with 10 ml of 0.4 M mannitol plus or minus $AgNO_3$, $Ca(NO_3)_2$, or KNO_3. Salt concn from 0.02 to 60 mM were employed. Glass beads were used to keep the cylinders immersed. The test tubes were placed in a 10 liter desiccator, which was evaluated 3 times to 25 mm Hg and maintained each time for 1 min. The cylinders were removed, blotted, re-weighed, placed in plastic gas-tight syringes which were set to 10 ml and capped with rubber serum stoppers. Gas samples were taken after 1 hr and analyzed for ethylene and CO_2 by gas chromatography.

Commercially obtained mature green banana fruits (*Musa* sp. cv. Valery) were surface-sterilized in Clorox (5.25% sodium hypochlorite) diluted 1:9 with water, and rinsed in sterile water. Although all fruit were mature green and selected for uniformity, each lot of fruit varies slightly in its degree of ripeness. All cutting, washing and other manipulations were

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performed in a bench-top transfer hood under aseptic conditions. One-cm thick slices were cut free-hand from the central portion of the banana and placed in sterile water for 10 min to remove latex and surface debris. The slices were blotted dry, placed in sterile, tared, plastic Petri dishes and weighed. Each slice was individually infiltrated with solutions similar to those used for apple tissue, by placing it flat on a layer of glass beads in a small jar with enough solution to cover it to a depth of 5 mm. The pressure was reduced 3 times to 25 mm Hg and maintained for 1 min each time while shaking the jar. The slice was removed, blotted dry, placed in its Petri dish and re-weighed. The Petri dishes, with covers ajar, were stacked in a disiccator and gassed with humid air containing 10 μl liter^{-1} ethylene for 4 to 6 days at a flow rate of ca. 300 ml min^{-1}. After flushing the desiccator with ethylene-free air for 4 hr, Petri dishes were removed, and the bottom dishes covered with a layer of Para-Film. Gas samples were taken after 1 hr and analyzed for ethylene and CO_2. The peel was visually rated for color development (yellow = 1, green = 7). The slice was placed flat on a bench and a thin layer of tissue cut away from the exposed surface. The pressure required to force a 1.5 mm diam plunger into the peel, perpendicular to the slice and about 3 mm from the cuticular surface was used as a measure of the firmness of the peel. Starch-to-sugar conversion was measured by homogenizing a known weight of pulp in 5 ml of water, centrifuging for 15 min at 3000 g, and measuring the soluble solids in the supernatant solution with a hand-held refractometer.

Mature green tomato fruits (*lycopersicon esculentum* Mill.) were obtained cmmercially, surface-sterilized and handled in an aseptic fashion similar to the banana tissue. Discs of pericarp tissue 1.5 cm diam were cut from locules with a cork borer. After blotting dry, the discs were dipped in various concn of $AgNO_3$, $Ca(NO_3)_2$, $CdCl_2$, $Cu(NO_3)_2$, $NiCl_2$, KNO_3, or $ZnCl_2$ for 1 to 2 min, drained and placed eipdermal surface down on a layer of 6 mm glass beads in a tared glass vial. The vials were capped with porous foam plugs, placed in desiccators, and gassed with ethylene in air for 4 to 6 days as previously described. After flushing the desiccators with ethylene-free air for 4 hr, the vials were removed, weighed, and the foam plugs replaced with rubber serum stoppers. Gas samples were taken after 2 hr and analyzed for ethylene and CO_2. Color changes associated with ripening were measured as reflectance at 650 nm (lycopene synthesis), or as decreased reflectance at 550 nm (chlorophyll destruction). Lycopene and chlorophyll contents were measured using the extraction procedure of Streif and Bangerth (15).

All experiments were repeated 3 times with 3 to 6 replicates per treatment. Each fruit was used as a block, with cylinders, slices, or discs of inidvidual fruit subjected to all treatments.

Results and Discussion

Infiltration of apple cortical cylinders with solutions of $AgNO_3$ above 0.03 mM significantly reduced ethylene production, but stimulated CO_2 production (Fig. 1). Respiration reached a maximum at 0.1 mM and declined to near zero above 2.0 mM. Solutions of KNO_3 or $Ca(NO_3)_2$ had no effect on ethylene or CO_2 production. The surface 1 mm of the cylinders infiltrated with 0.1 mM $AgNO_3$ turned light brown in color, but the tissue remained turgid and the interior was not discolored. Higher concn of $AgNO_3$ caused the tissue to become flacid and internally discolored. The decline in ethylene and CO_2 production above 0.5 mM $AgNO_3$ can probably be attributed to acute toxicity and death.

Several parameters of banana ripening were not inhibited at concn of Ag(I) which severely inhibited others. The rate of ethylene production was significantly reduced in banana fruit slices which had been infiltrated with solutions of $AgNO_3$ above 0.3 mM (Fig. 2) or 0.03 mM (Table 1), while CO_2

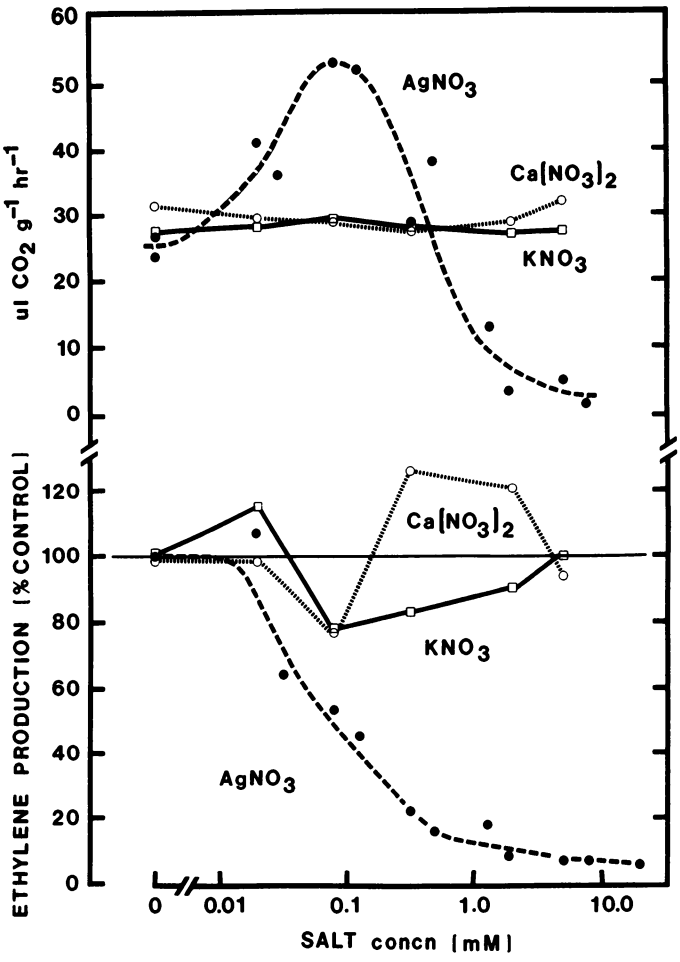

Fig. 1. Effect of various salt concn on ethylene and CO_2 production by 3 cm by 1 cm diam cortex cores of post-climacteric apple fruit during the hr immediately following infiltration.

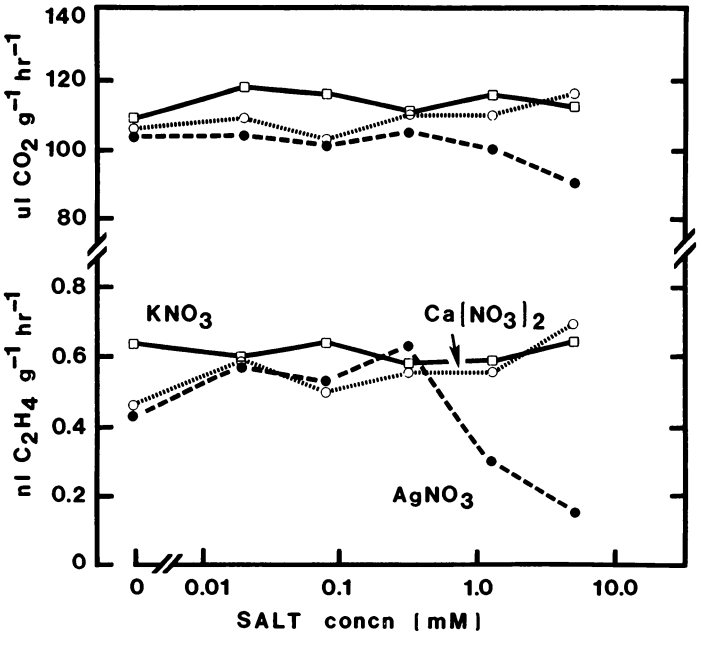

Fig. 2. Effect of various salt concn on ethylene and CO_2 production by 1-cm thick slices of mature green banana fruit gassed with 10 μl liter^{-1} ethylene in air for 4 days.

Table 1. Effect of infiltration with $AgNO_3$ on various ripening parameters of banana fruit.

$AgNO_3$ treatment (mM)	Color[x] index	Firmness (g/mm^2)	Soluble solids (%)	Ethylene (nl/g-hr)	CO_2 (μl/g-hr)
0.00	1.4 a[z]	28.8 a	9.7 a	4.81 a	134
0.03	1.4 a	44.6 a	9.4 a	2.51 b	85
0.06	3.2 b	117.5 b	9.4 a	2.97 b	113
0.12	3.6 b	198.9 c	8.3 a	1.89 b	83
0.24	4.8 c	197.7 c	8.1 a	2.02 b	107
0.48	5.2 c	198.3 c	8.0 a	2.34 b	135
0.96	6.0 cd	198.3 c	7.5 ab	1.47 c	109
1.92	6.8 d	195.5 c	5.2 b	0.43 d	89

[x]Color index for peel; yellow = 1, green = 7.

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

production was unaffected (Table 1) or slightly reduced (Fig. 2). The variable tissue response may have been the result of slight differences in fruit ripeness in different lots of bananas. Because of the variability, salt concn from 0.02 to 60 mM were used to insure that the optimal treatment concn was within the concn range. Although yellowing and softening of the banana peel was reduced at a concn of 0.06 mM $AgNO_3$, a 15- to 30-fold higher concn was required to inhibit accumulation of soluble solids in the pulp due to starch hydrolysis (Table 1). Examination of slices showed that, whereas the silver solution easily penetrated the peel, the pulp was almost impervious to its penetration. Since the majority of ethylene and CO_2 production occurs in the pulp (Table 2), it is not surprising that higher concn of silver were required to reduce synthesis by this tissue. Division of whole slices into pulp and peel probably stimulated ethylene and CO_2 production by increasing gas diffusion into the tissue, and by physically wounding the tissue. Solutions of KNO_3 or $Ca(NO_3)_2$ had no effect on ethylene or CO_2 production (Fig. 2).

Silver nitrate between 0.1 and 5 mM significantly reduced ethylene and CO_2 production by pericarp discs of tomato, but rates of production increased to the control level at 20.5 mM (Fig. 3). Such high concn of silver nitrate were phytotoxic as evidenced by tissue discoloration and loss of turgor. Toxic concn of chemicals have been shown to induce wound respiration and stimulate ethylene synthesis (1, 7, 14). Reflectance values depicting chlorophyll retention (Fig. 3) and measured chlorophyll content (Fig. 4) were increased, and lycopene content (Fig. 4) was decreased at concn of silver between 0.2 and 1.3 mM. Solutions of KNO_3 or $Ca(NO_3)_2$ had no effect on reflectance of CO_2 production (Fig. 3). Ethylene production was not affected by KNO_3, but was slightly stimulated by $Ca(NO_3)_2$ solutions. Of the 7 metallic salts tested, only $AgNO_3$ reduced red pigment development at a concn (0.1 mM) which did not cause visual signes of tissue necrosis (Fig. 5).

Attempts to infiltrate solutions into pericarp discs of tomato at 50 or 150 mm Hg caused abnormal ripening in all treatments

Table 2. Production of ethylene and CO_2 by whole slices and slices divided into peel and pulp of mature green banana fruit.

Tissue	Rates of production: C_2H_4 (nl/g-hr)	Rates of production: CO_2 (μl/g-hr)
Whole slices	0.78 a[z]	190 a
Peel	2.20 b	210 a
Pulp	5.01 c	330 b

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

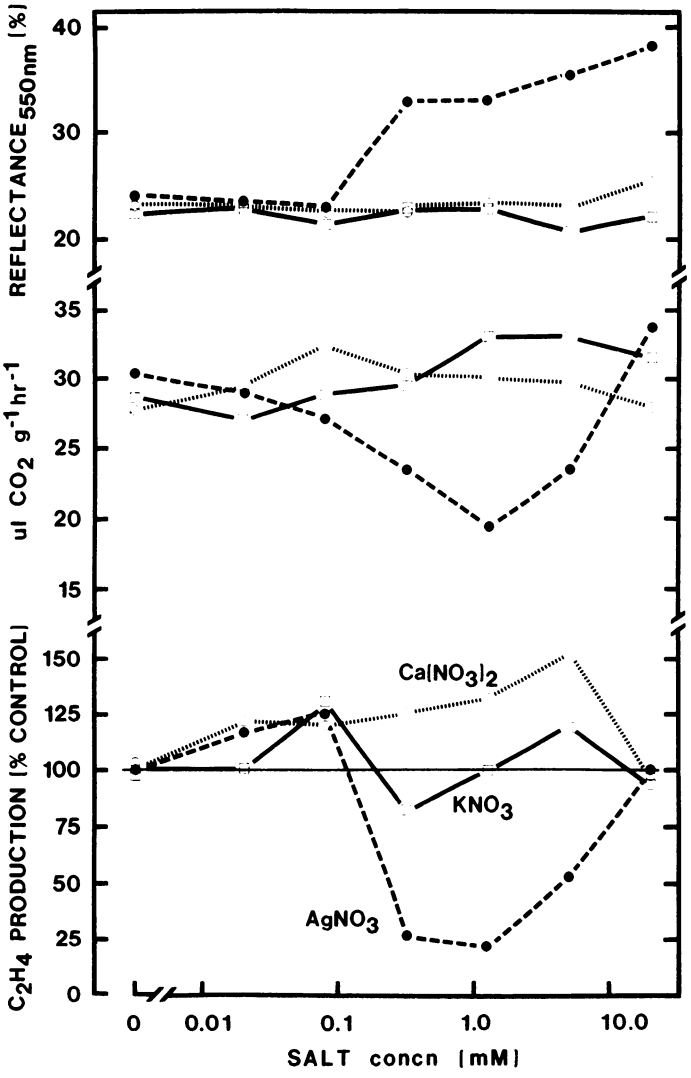

Fig. 3. Effect of various salt concn on ethylene and CO_2 production, and on reflectance at 550 nm by 1.5 cm diam pericarp disks of mature green tomato fruit gassed with 10 μl liter^{-1} ethylene for 4 days. Higher reflectance values indicate lower lycopene content.

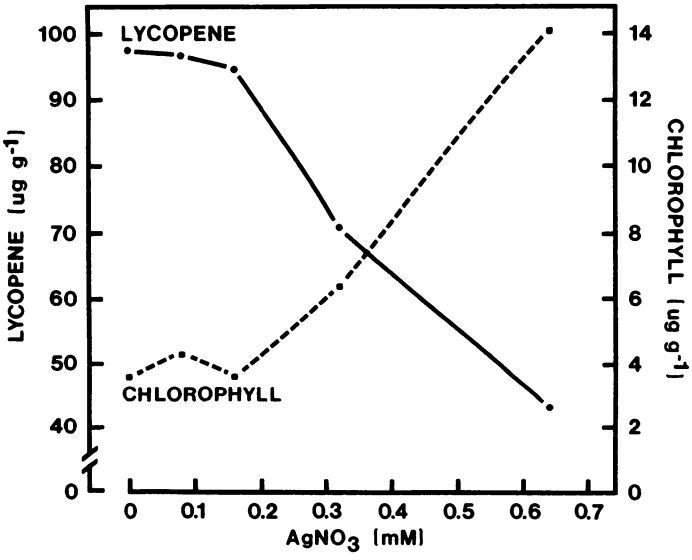

Fig. 4. Effect of $AgNO_3$ concn on chlorophyll degratation and lycopene synthesis by 1.5 cm diam pericarp disks of mature green tomato fruit gassed with 10 μl liter^{-1} ethylene for 4 days.

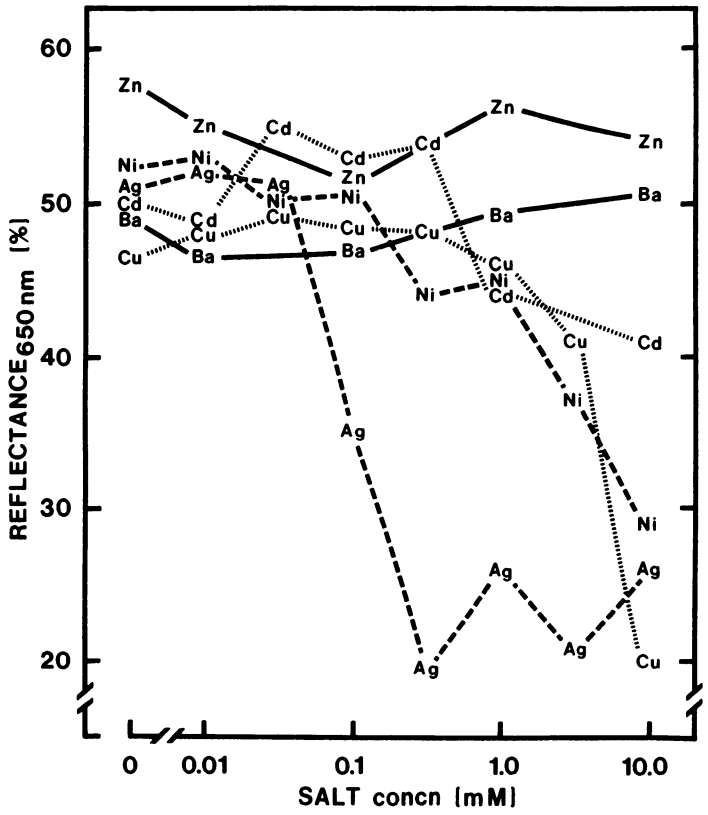

Fig. 5. Effect of various concn of metallic salts on reflectance at 650 nm by 1.5 cm diam pericarp disks of mature green tomato fruit gassed with 10 μl liter^{-1} ethylene for 4 days. Higher reflectance values indicate greater red color development.

(data not shown). A 1 to 2 min dip in the solutions proved most effective in allowing normal ripening. However, limited penetration of the solutions from the epidermal surface of the disc prevented the salts from affecting a lens shaped region of tissue adjacent to the epidermis.

Silver ions were clearly the most effective of 7 metal ions tested in inhibiting ethylene synthesis and ripening in the 3 climacteric species of fruit examined. The inability of applied ethylene to elicit normal ripening in silver treated fruit tissue suggests that Ag(I) interferes with an early and initially critical step in ethylene action in the ripening sequence. If Ag(I) acts by blocking receptor sites for ethylene perception, and thereby reduces the tissue sensitivity to ethylene, then we would expect to see the observed titration of the various ripening parameters with increasing Ag(I) concn as shown in Table 1 and Fig. 1, 4, and 5. Although our data does not identify the site(s) of inhibition by silver, it agrees with other published papers (2, 8, 9) in indicating that sensitivity to ethylene, ethylene production, and ripening are closely related. Tissue heterogeneity and nonuniform infiltration of Ag(I) solution in banana and tomato tissues probably are the causes for the observed differential effect of Ag(I) on the various ripening parameters. However, multiple mechanisms of ethylene action in plants, as proposed by Goesehl and Kays (6), cannot be ruled out.

Although the toxicity of silver precludes its use to extend the storage life of food crops, it may be a useful tool with which to differentiate and investigate various aspects of the ripening process.

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