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A Potent Inhibitor of Ethylene Action in Plants[1]

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ABSTRACT

Ag(I), applied foliarly as $AgNO_3$, effectively blocked the ability of exogenously applied ethylene to elicit the classical "triple" response in intact etiolated peas (*Pisum sativum* cv. Alaska); stimulate leaf, flower, and fruit abscission in cotton (*Gossypium hirsutum* cv. Stoneville 213); and induce senescence of orchids (Hybrid white *Cattleya*, Louise Georgeianna). This property of Ag(I) surpasses that of the well known ethylene antagonist, CO_2, and its persistence, specificity, and lack of phytotoxicity at effective concentrations should prove useful in defining further the role of ethylene in plant growth.

Ethylene is widely recognized as an important ubiquitous plant hormone involved in many developmental processes including fruit ripening, abscission, senescence, growth, flowering, and sex expression (1). However, the initial biochemical event triggered by ethylene which ultimately causes these diverse developmental changes is unknown. During studies (3, 5, 6) aimed at better understanding this key event, Ag(I) was found to block ethylene action effectively. This property of Ag(I) surpasses that of the well known ethylene antagonist, CO_2 (1), and therefore, should provide a new experimental tool for studying ethylene action and defining further its role in plant growth. Here, the author reports the ability of Ag(I) to block specifically the action of exogenously applied ethylene in such classical ethylene responses as abscission, senescence, and growth retardation.

MATERIALS AND METHODS

The general experimental approach was to treat foliarly intact pea, cotton, and orchid plants with various concentrations of $AgNO_3$ and observe the protection afforded by such treatments following a given ethylene exposure. Although silver nitrate was the preferred salt, because of its water solubility and immediate laboratory availability, other salts such as silver acetate and silver lactate have also been found to be effective. A variety of other metal ions including Ni, Hg, Co, Cd, Cu, Pd, Pt, Rh, Zn, and Ru were evaluated for their ability to block ethylene action, but none were effective when applied foliarly to intact plants with the exception of palladium (K_2PdCl_4) and mercury ($HgNO_3$ and $Hg(NO_3)_2$, whose effects were marginal.

Alaska peas were planted in vermiculite in 10-cm plastic pots, watered with distilled H_2O, and grown for 4 days in a dark room at a constant 23 C and 75% relative humidity. Using a "safe" green light, individual pots containing approximately 10 seedlings were treated foliarly with 0, 15, 60, or 240 mg/liter of $AgNO_3$ containing 0.1% Tween 20 as a surfactant. Two hr after treatment, control (solvent-treated) and treated pots were placed in two dark chambers. One chamber was purged with air at 15 liters/min, while the other was purged at the same flow rate with 0.25 μl/liter of ethylene.

Cotton plants (*Gossypium hirsutum* cv. Stoneville 213), were grown in an environmental growth room as previously described (4), and at 5 weeks of age, they were treated with aqueous solutions containing 0, 25, 50, 100, or 200 mg/liter of $AgNO_3$ plus 0.01% Tween 20 as a surfactant. The following day, treated and control plants were placed in two large chambers (4). One chamber was purged with 12 μl/liter of ethylene at 18 liters/min, while the other was purged with air at the same flow rate.

Hybrid white *Cattleya* Louise Georgeianna orchids were purchased from a nearby commercial greenhouse grower. Upon delivery, the bare rooted plants were immediately potted in 100% medium grade Douglas fir bark in 17.6-cm plastic pots, watered with tap water, and placed in an environmental growth room (27-C day, 21-C night; 1400 ft-c; 16-hr photoperiod; 62 to 68% relative humidity). About 3 days prior to flower scape emergence from the sheath, the entire plant was treated once each day with 750 mg/liter of $AgNO_3$ containing 0.01% Tween 20 as a surfactant. As soon as the flower scape emerged from the sheath, the $AgNO_3$ concentration was dropped to 10 mg/liter to avoid injury to the delicate sepals. The entire flower scape was sprayed to run off each day with this reduced amount of $AgNO_3$ until the individual flowers were fully open. This generally required 2 to 3 days. Then, both control (solvent-treated) and treated plants with fully opened flowers were placed in a large chamber in the laboratory and exposed to 0.2 μl/liter of ethylene for 24 hr. Plants were then returned to the environmental growth room and observed daily. It was necessary to expose the flowers to ethylene shortly after they had fully opened since 3 to 5 days later, flower hardening had occurred which greatly reduced their sensitivity to ethylene.

RESULTS

Figure 1 shows the general appearance of control and $AgNO_3$-treated pea seedlings after 3 days in ethylene or air. The control seedlings in ethylene exhibited the classical "triple" response, which included growth retardation, stem swelling, and horizontal growth. Increasing concentrations of $AgNO_3$ progressively and uniformly reduced these characteristic ethylene effects. As shown in Figure 1, almost complete protection from the effects of ethylene occurred with a treatment of 240 mg/liter of $AgNO_3$. This degree of protection is indeed amazing considering the multiplicity of ethylene responses in pea. The effect of Ag(I) is systemic since the new growth which occurred during the 3-day exposure to ethylene was also protected from ethylene.

Figure 2 illustrates the dramatic and unique ability of Ag(I) to block ethylene-stimulated leaf abscission in cotton. Without $AgNO_3$ treatment, all of the leaves had abscised by the 7th day in ethylene (Fig. 2, extreme left), while none had abscised in the corresponding control plants placed in air (Fig. 2, extreme right). Plants treated with increasing concentrations of $AgNO_3$

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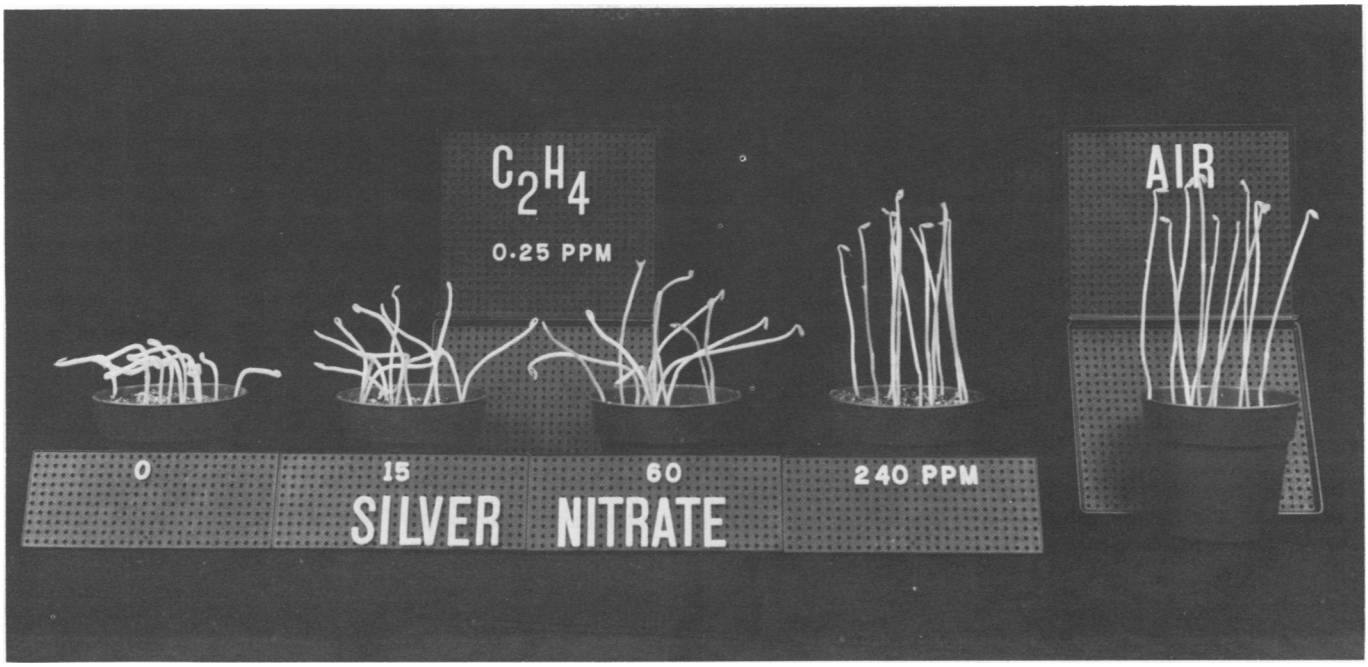

FIG. 1. Prevention of ethylene-induced growth changes in pea by $AgNO_3$ applied as an initial foliar treatment 2 hr before a continous 3-day exposure to 0.25 μl/liter of ethylene. Pot on extreme right contains seedlings treated with 240 mg/liter of $AgNO_3$ and placed in air. Control seedlings in air were slightly shorter, being about equal in height to the 240 mg/liter $AgNO_3$-treated seedlings in ethylene. Plants treated with 240 mg/liter of $NaNO_3$, and placed in ethylene, were identical to the untreated ethylene controls on the extreme left, thus eliminating any possible anion effect.

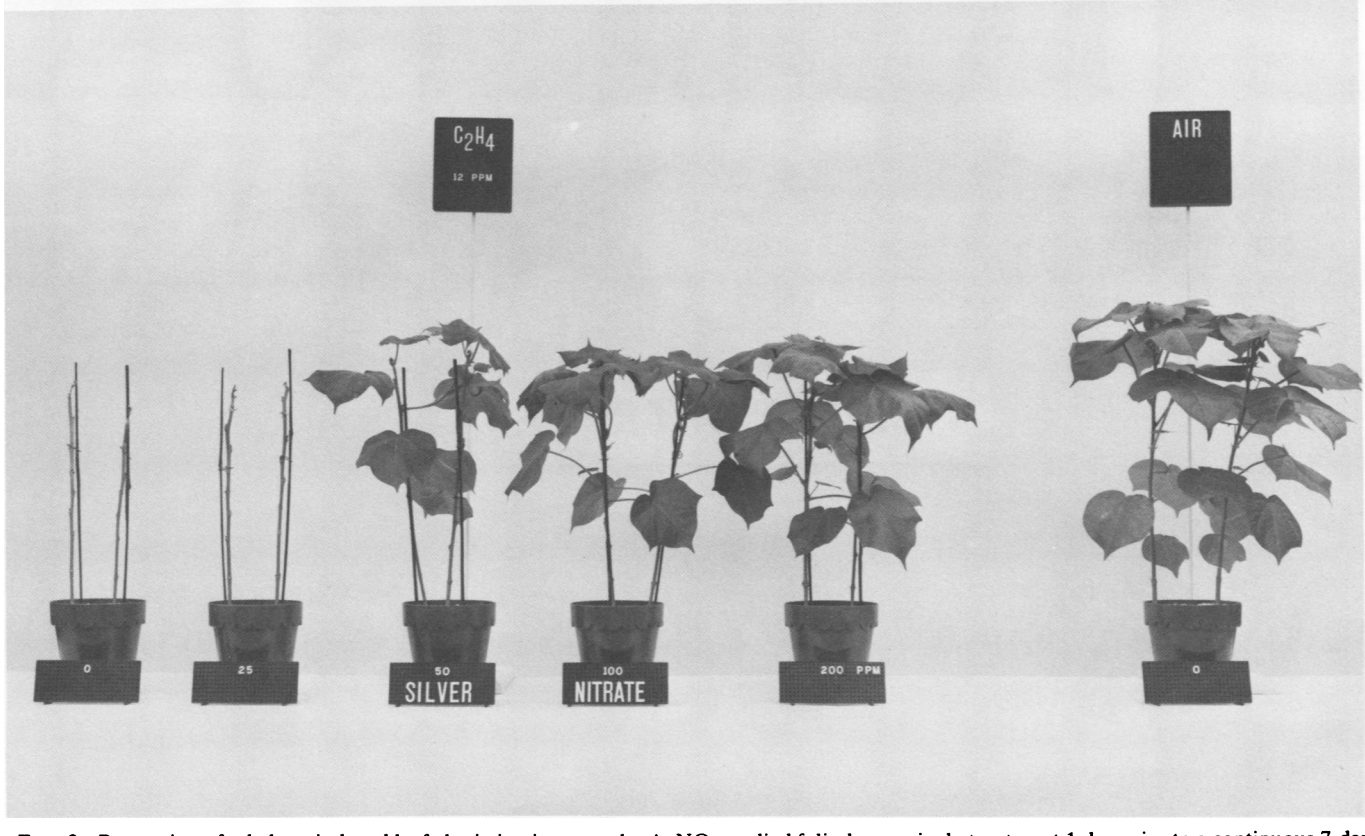

FIG. 2. Prevention of ethylene-induced leaf abscission in cotton by $AgNO_3$ applied foliarly as a single treatment 1 day prior to a continuous 7-day exposure to 12 μl/liter of ethylene. Pot on right contains the control or solvent-treated plants which did not receive ethylene. No major growth differences were observed between these air control plants and those treated with 200 mg/liter of $AgNO_3$ and left in air during the 7 days.

and placed in ethylene showed progressively less leaf abscission. Treatment with 25 mg/liter of $AgNO_3$ reduced the time required to reach 100% leaf abscission by 2 days. However, as shown in Figure 2, by the 7th day, all of the leaves on these plants, like the untreated plants in ethylene, had abscised. In contrast, total leaf abscission was only 50, 35, or 9% in plants pretreated with a single application of 50, 100, or 200 mg/liter of $AgNO_3$, respectively. Considering the level of ethylene (12 μl/liter) and the

prolonged exposure time (7 days), this was a remarkable degree of protection. As shown by the height of the plants in Figure 2, $AgNO_3$ treatment also slightly reduced the growth-retarding effect of ethylene. Other similar experiments with mature fruiting cotton plants have demonstrated a similar ability of $AgNO_3$ to prevent young fruit and flower abscission.

Figure 3 illustrates the dramatic protective effect that $AgNO_3$ has in preventing ethylene-induced flower senescence in orchids. Five days after a 24-hr ethylene exposure, the control flowers were dead, whereas those treated with $AgNO_3$ were in excellent condition. The concentration of $AgNO_3$ applied directly to the flower was critical since too high a rate caused sepal damage, while too low a rate did not provide adequate protection. In these experiments, 15 mg/liter of $AgNO_3$ caused slight damage when applied directly to the flower while 10 mg/liter of $AgNO_3$ did not.

DISCUSSION

The ability of a substance to block specifically the action of a plant hormone in the intact plant to the extent reported here is unparalleled in plant biology. The most outstanding antiethylene properties of Ag(I) are its persistence, specificity, and its lack of phytotoxicity at effective concentrations. In addition to the responses reported here, Ag(I) has also been found to block ethylene action in tomato and cucumber plants. Although the basis for this protection by Ag(I) is unknown, it is not due to a general scavenging for ethylene by Ag(I), since the concentration of ethylene entering and leaving the chamber was always the same. Furthermore, Ag(I) does not irreversibly bind ethylene, and is a much less effective trap for ethylene than Hg(II), for example, which has only a marginal effect in blocking ethylene action. In contrast to the weak ethylene antagonist, CO_2 (1), which shows classical competitive inhibition kinetics by Lineweaver-Burk-type plots, Ag(I) shows noncompetitive behavior (E. M. Beyer, Jr. unpublished data).

Previously, the author suggested that the oxidation of ethylene to CO_2, which comprises one aspect of the ethylene metabolism system in pea, may be closely linked to its mechanism of action (5, 6). As currently envisioned, this oxidation may involve a metal-ion enzyme system. The basis for the existence of a metallic receptor site for ethylene in plants and the involvement of O_2, as well as the antagonistic effect of CO_2 has been reviewed (1) and recently discussed in terms of the ethylene metabolism-ethylene action hypothesis (6). Earlier studies (2, 3) with deuterated ethylene do not refute the metal receptor site concept for reasons already discussed (3). Cu(I) is proposed as the metal involved, since Cu(I) is known to form complexes with ethylene

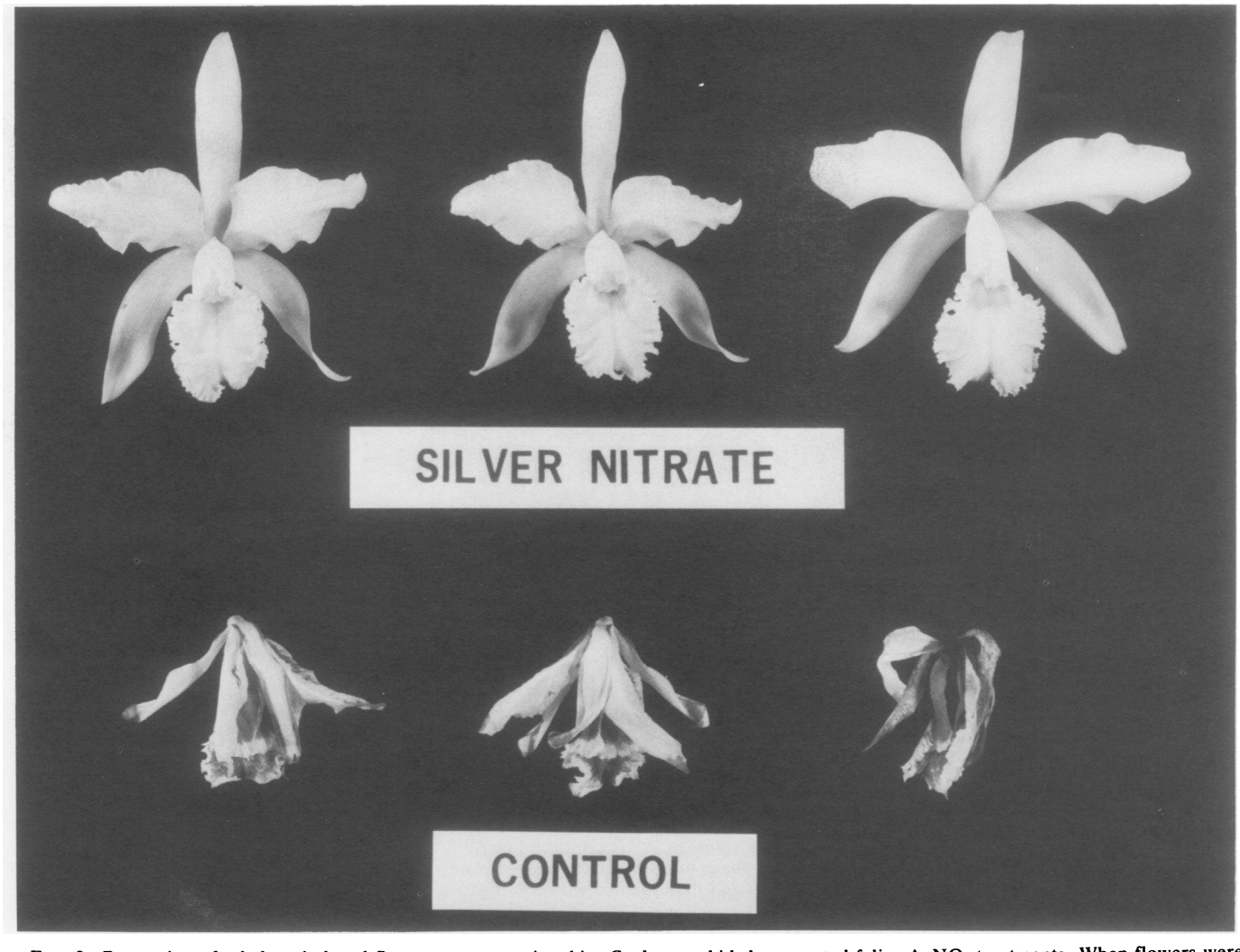

FIG. 3. Prevention of ethylene-induced flower senescence in white *Cattleya* orchids by repeated foliar $AgNO_3$ treatments. When flowers were fully opened, solvent control and $AgNO_3$-pretreated plants were exposed to 0.2 μl/liter of ethylene for 24 hr. Flowers shown were excised from treated and control plants 5 days following ethylene exposure.

(7). In contrast, other metals commonly found in biological systems such as Fe(II) and Fe(III) are less likely to do so (7). In this system, the effect of Ag(I) could be explained on the basis that Ag(I) substitutes for Cu(I), thereby interfering with ethylene oxidation, and hence ethylene action. The similarity in size, the same oxidation state, and the ability of both Ag(I) and Cu(I) to form complexes with ethylene (7) lend credence to this idea. This possibility, as a basis for the antiethylene property of silver, is currently being explored.

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