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Light Requirement for $AgNO_3$ Inhibition of Ethrel-Induced Leaf Abscission from Cuttings of *Vigna radiata*[1]

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ABSTRACT

To obtain information regarding the antiethylene properties and binding site of Ag^+, studies were initiated to define conditions under which Ag^+ does or does not inhibit ethylene action. $AgNO_3$, applied as a leaf spray, inhibited 2-chloroethylphosphonic acid (Ethrel)-induced leaf abscission from green cuttings of *Vigna radiata* in white light but lost considerable activity in the dark. In the absence of Ethrel, $AgNO_3$ stimulated abscission in the dark. When cuttings were dark-aged for 24 hours prior to treatment with $AgNO_3$ and aged for an additional 24 hours in the dark after treatment, good inhibition of subsequent Ethrel-induced abscission was restored by returning the cuttings to light. However, when dark aging was preceded by far-red irradiation, considerably less inhibition of Ethrel-induced abscission was restored in the light. $AgNO_3$ was completely inactive on cuttings aged in the dark and treated with Ethrel in the dark. Light is required for the antiethylene activity of $AgNO_3$ with regard to leaf abscission of *Vigna*.

Ag^+ is an effective inhibitor of several physiological responses mediated by ethylene, such as the triple response, abscission, senescence (1, 4, 5, 9, 12, 14), rolling up of flower rib segments (2), cytokinin accumulation in reproductive organs (13), increases in chlorophyllase levels, and destruction of chloroplast membranes (11). Although the mode of action of Ag^+ in inhibiting ethylene action has not been established, it was suggested that Ag^+ may attach to the ethylene binding site (1, 4). Because Cu^+ is believed to be involved at the ethylene binding site (3), it was suggested that Ag^+ might substitute for Cu^+ (4), but this suggestion has been questioned (5). The possibility that two receptor sites for ethylene are present has been raised (2, 5).

If it is assumed that Ag^+ inhibits ethylene action by attachment to the ethylene binding site, then studies which define conditions under which Ag^+ does or does not inhibit ethylene action might provide information regarding the nature of the site. Furthermore, the ability of Ag^+ to inhibit ethylene action might also provide a method for assessing the role of ethylene in physiological processes. For this reason, I examined the effect of Ag^+ on leaf abscission in mung bean (*Vigna radiata*) in the presence and absence of Ethrel (2-chloroethylphosphonic acid), an ethylene-releasing compound, and in the light and dark.

MATERIALS AND METHODS

Cuttings of *Vigna radiata* (L.) Wilczek cv. Jumbo were used for leaf abscission studies, as described previously (7). Approximately 75 seeds were sown in plastic cartons (11.5 cm o.d. × 8 cm deep) containing vermiculite and grown in the greenhouse for 12 to 14 days. Cuttings were excised 8.0 cm from the base of the apical bud, transferred randomly to 12-ml vials containing approximately 10 ml deionized H_2O (10 cuttings/vial), sprayed to run-off with 0.1% Tween 20 with and without $AgNO_3$, and incubated in continuous white fluorescent light (Champion F90T17/w, 4×10^3 ergs/cm^2·s, 27–29°C). After 24 h, cuttings were transferred to vials containing H_2O or solutions of Ethrel and incubated in continuous white fluorescent light (1.35×10^4 ergs/cm^2·s) or in the dark at 28°C. Deionized H_2O was added daily to maintain the volume at 10 ml. Abscission of the primary leaves was determined daily after rotating the stems rapidly between the fingers. Experiments performed in the dark were evaluated under a dim green safelight. Treatments were not identified until after abscission had been determined. Each vial was considered a single determination. Most experiments were performed three times and consisted of three to four determinations per treatment.

FR[2] radiation was supplied by a 250-w internal reflector incandescent bulb and passed through an 8- to 10-cm-deep water bath placed above a portable growth chamber containing a FR filter (750 nm, Carolina Biological Supply, Burlington, NC). Irradiance was 1.8×10^4 ergs/cm^2·s, and irradiations were for 10 min.

RESULTS

To determine if $AgNO_3$ inhibited Ethrel-induced abscission in *Vigna*, cuttings sprayed with $AgNO_3$ and maintained for 24 h in light were transferred to vials containing Ethrel (1.38 mM) and incubated in the light. Abscission was determined after 2 days. $AgNO_3$, in concentrations ranging from 0.125 to 1.0 mM, was an effective inhibitor of Ethrel-induced abscission (Fig. 1). When solutions of Ethrel and $AgNO_3$ were mixed and applied to the base of cuttings, $AgNO_3$ had little or no effect on abscission rates (results not given). Because addition of 1.0 mM $AgNO_3$ to a 1.38 mM Ethrel solution resulted in a milky, opaque solution, $AgNO_3$ could have been "inactivated." However, when such a solution was used as a leaf spray and the cuttings were incubated for 24 h in light, no decrease in the ability of Ag^+ to inhibit Ethrel-induced abscission in the light was observed.

When cuttings of *Vigna* were placed in the dark, almost all leaves abscised within 5 to 7 days. To determine the role, if any, of ethylene in dark abscission, cuttings were sprayed, incubated in light for 24 h, and transferred to the dark. Depending on the concentration, $AgNO_3$ either stimulated (1.0 mM) or had no effect on dark abscission (Fig. 2). When $AgNO_3$ was applied to the basal end of the cuttings and the cuttings were placed immediately in the dark, an even greater stimulation of abscission occurred (Fig. 2).

FR irradiation prior to dark incubation stimulates leaf abscission from cuttings of *Vigna* relative to nonirradiated controls (9). The effect of $AgNO_3$ on enhancement of dark abscission by FR

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[2] Abbreviation: FR, far red.

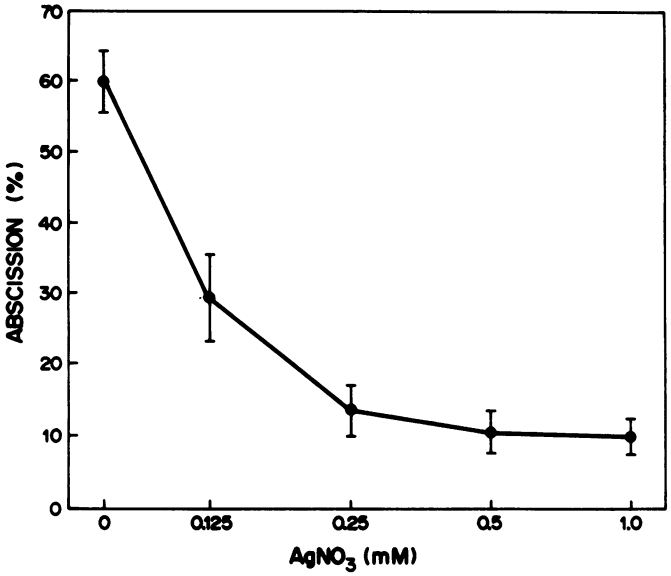

FIG. 1. Effect of $AgNO_3$ on Ethrel-induced abscission by cuttings of *Vigna* in white light. Cuttings sprayed with $AgNO_3$ and incubated for 24 h in light prior to basal application of 1.38 mM Ethrel. Abscission determined after 48 h in light. Means of nine determinations ±SE.

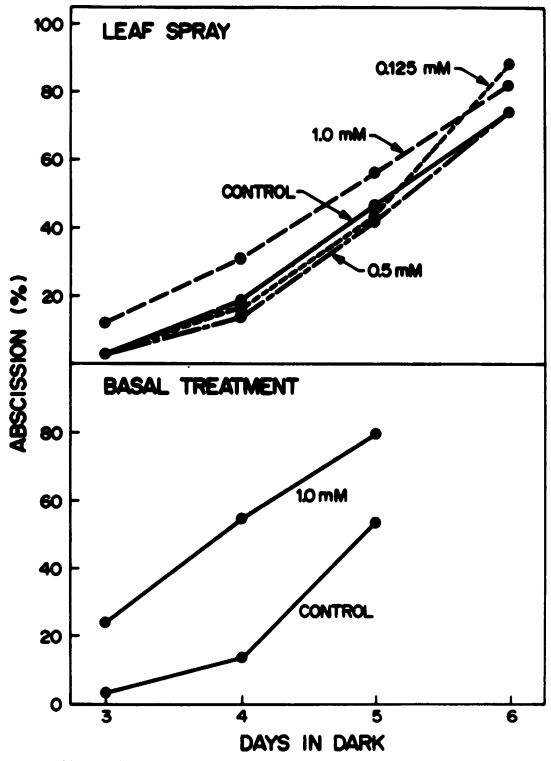

FIG. 2. Effect of $AgNO_3$ on dark abscission by cuttings of *Vigna*. Top, cuttings sprayed with $AgNO_3$, incubated for 24 h in light, and transferred to the dark. Bottom, cuttings placed in vials containing $AgNO_3$ and placed immediately in the dark.

was determined with cuttings which had been sprayed, incubated for 24 h in light, and placed in the dark with and without a 10-min FR treatment (Fig. 3). $AgNO_3$ did not modify the effect of FR on abscission rates.

Enhanced ethylene production could account for stimulation of dark abscission by cuttings previously sprayed with $AgNO_3$ (Fig. 2). If so, then addition of malformin should potentiate the action

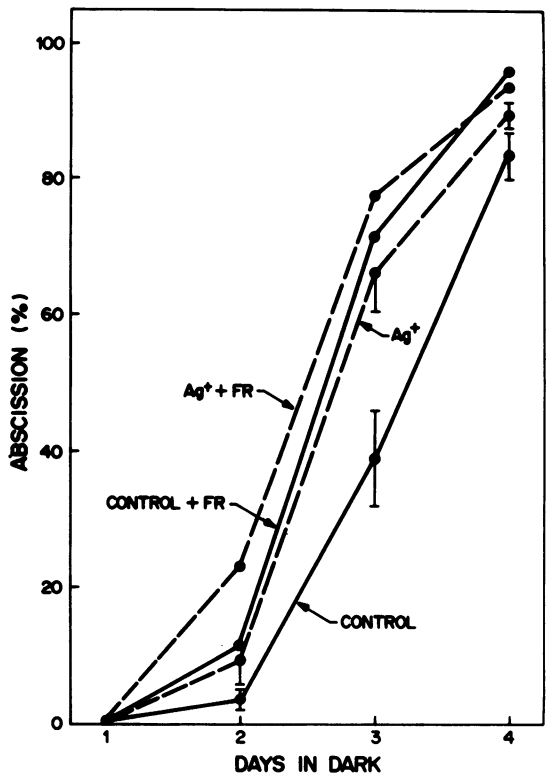

FIG. 3. Effect of $AgNO_3$ on stimulation of dark abscission by FR irradiation. Cuttings sprayed with 0.1% Tween 20 or 1.0 mM $AgNO_3$, incubated in light for 24 h, and placed in dark with and without prior irradiation with FR light (10 min).

Table I. *Effect of Malformin on Enhancement of Dark Abscission by $AgNO_3$ and Ethrel*

Cuttings were sprayed with 0.1% Tween 20 or 1.0 mM $AgNO_3$, incubated for 24 h in light, transferred to vials with and without 1.0 μM malformin or 13.8 μM Ethrel, and placed in the dark. Abscission was determined after 48 h. Means of eight determinations ± SE.

Treatment		Abscission
Light	Dark	
		%
Tween 20	H_2O	0
$AgNO_3$	H_2O	3.2 ± 1.5
Tween 20	Malformin	6.0 ± 3.0
$AgNO_3$	Malformin	24.0 ± 3.5
Tween 20	Ethrel	14.0 ± 2.0
Tween 20	Ethrel + malformin	54.0 ± 7.5

of Ag^+, because malformin potentiates ethylene-induced abscission (6). Cuttings previously sprayed with 1.0 mM $AgNO_3$ and incubated for 24 h in light were transferred to the dark in the presence and absence of 1.0 μM malformin. For comparison, cuttings which had been sprayed with Tween 20 only were transferred to the dark into vials containing 13.8 μM Ethrel with and without 1.0 μM malformin. Malformin potentiated both Ag^+- and Ethrel-induced abscission (Table I).

Failure of Ag^+ to inhibit dark abscission required an assessment of the efficacy of Ag^+ to inhibit Ethrel-induced abscission in the dark. Cuttings were sprayed, incubated for 24 h in light, transferred to vials containing 1.38 mM Ethrel, and placed in the dark with and without prior FR irradiation. For comparison, other cuttings were treated with Ethrel in light. The ability of Ag^+ to inhibit Ethrel-induced abscission was markedly reduced in the

dark (Table II). For example, after 2 days, Ag^+ inhibited abscission 92% in light but only 21% in the dark. FR irradiation prior to dark incubation reduced inhibition by Ag^+ further (12.5%), but the difference between irradiated and nonirradiated cuttings was not great.

In the previous experiments, $AgNO_3$ was applied as a spray, and the cuttings were maintained in continuous light for 24 h prior to treatment with Ethrel. For comparison, cuttings were sprayed, allowed to dry for approximately 30 min, and incubated in light, in dark, or in dark immediately following FR irradiation. After 24 h, cuttings were treated with Ethrel and incubated in light or in the dark (Table III). Abscission by cuttings treated in light with both Ag^+ and, subsequently, with Ethrel was inhibited about 90% by Ag^+, but Ag^+ had almost no effect on cuttings treated exclusively in the dark. However, when cuttings were treated with Ag^+ in the dark for 24 h and returned to light for Ethrel treatment, Ag^+ inhibited abscission approximately 75%. Although FR irradiation enhanced abscission on both treated and control cuttings, the relative amount of abscission was similar.

The efficacy of Ag^+ as an Ethrel antagonist on cuttings previously aged in the dark, or irradiated with FR and then aged in the dark, was also examined. One set of cuttings was placed in the dark for 24 h, sprayed with Tween 20 or $AgNO_3$, incubated for an additional 24 h in the dark, transferred to vials containing Ethrel, and placed in light or dark (treatment A). Another set was irradiated with FR for 10 min, placed in the dark for 6 h, sprayed with Tween 20 or $AgNO_3$, maintained in the dark for 18 h, transferred to vials containing Ethrel, and placed in the light or dark (treatment B). Ag^+ was completely inactive on cuttings incubated exclusively in the dark (Table IV, treatment A), but good inhibition (78%) of Ethrel-induced abscission was restored when cuttings were returned to light. FR-irradiated cuttings (Table IV, treatment B) were also unresponsive to Ag^+ when treated with Ethrel in the dark, and only modest restoration of Ethrel inhibition (23%) occurred in light.

Table II. *Effect of $AgNO_3$ on Ethrel-Induced Abscission by Cuttings of Vigna in the Dark*

Cuttings were sprayed with 0.1% Tween 20, with and without 1.0 mM $AgNO_3$, incubated in light for 24 h, transferred to vials containing 1.38 mM Ethrel, and placed in the dark with and without prior irradiation (10 min) with FR. For comparison, the effect of Ag^+ in light is included. Means of 10 determinations ±SE.

Treatment	Abscission			
	1 Day	2 Days	3 Days	4 Days
		%		
In light				
Tween 20	1.2 ± 1.2	97.0 ± 1.5	100.0	100.0
$AgNO_3$	0	7.5 ± 1.0	13.0 ± 2.5	15.0 ± 2.0
In dark				
Tween 20	34.5 ± 8.5	100.0	100.0	100.0
$AgNO_3$	11.5 ± 4.5	79.0 ± 4.5	98.0 ± 1.0	100.0
In dark (FR-irradiated)				
Tween 20	40.5 ± 7.5	100.0	100.0	100.0
$AgNO_3$	17.4 ± 7.0	87.5 ± 3.0	98.5 ± 1.0	100.0

Table III. *Effect of Light, Dark, and FR Irradiation on ability of $AgNO_3$ to inhibit abscission induced by subsequent treatment with Ethrel*

Cuttings were sprayed with 0.1% Tween 20 (control) or 1.0 mM $AgNO_3$, dried for 30 min, and incubated in light, dark, or in the dark after FR irradiation (10 min). After 24 h, cuttings were transferred to vials containing 1.38 mM Ethrel and incubated in light or dark. Average of 12 determinations ±SE.

Treatment	Abscission	
	1 Day	2 Days
	%	
24-h light-Ethrel-light		
Control	3.0 ± 1.5	99.0 ± 0.5
$AgNO_3$	0.4 ± 0.4	9.0 ± 2.5
24-h dark-Ethrel-dark		
Control	50.0 ± 11.0	100.0
$AgNO_3$	38.5 ± 7.0	91.5 ± 4.0
24-h dark-Ethrel-light		
Control	15.0 ± 7.0	99.0 ± 0.5
$AgNO_3$	13.0 ± 4.0	25.0 ± 4.0
FR-24-h dark-Ethrel-dark		
Control	83.5 ± 5.5	100.0
$AgNO_3$	64.0 ± 12.0	96.5 ± 2.0

DISCUSSION

When sprayed on leaf surfaces, $AgNO_3$ is an effective inhibitor of Ethrel-induced leaf abscission on cuttings of *Vigna* in white light (Fig. 1). Failure of $AgNO_3$ to inhibit abscission when applied basally is probably due to slow acropetal movement of Ag^+. In cut carnations, Ag^+ as $AgNO_3$ moves upward about 3 cm per day (14). If a similar rate of movement applies to *Vigna*, which had 8.0-cm-long stems, Ag^+ probably arrived at effective receptor sites too late to inhibit Ethrel-induced abscission.

One aim of this investigation was to employ Ag^+ as a tool to examine the role, if any, of ethylene in dark abscission. When cuttings previously sprayed with Ag^+ in light were treated with Ethrel and transferred to the dark, inhibition of Ethrel-induced abscission was markedly reduced (Table II). Although significant inhibition of Ethrel action was observed in the dark, light is

Table IV. *Ability of $AgNO_3$ to Inhibit Ethrel-Induced Abscission on Cuttings of Vigna Previously Aged in the Dark With and Without FR Irradiation*

Treatment A: cuttings were aged for 24 h in dark, sprayed with 0.1% Tween 20 (control) or 1.0 mM $AgNO_3$, aged an additional 24 h in the dark, transferred to vials containing 1.38 mM Ethrel, and placed in light or dark. Treatment B: cuttings were irradiated with FR (10 min), placed in dark for 6 h, sprayed as above, aged an additional 18 h in the dark, transferred to vials containing Ethrel, and placed in light or dark.

	Abscission	
	1 Day	2 Days
	%	
Treatment A		
Dark		
Control	100.0	
$AgNO_3$	99.0 ± 0.5	
Light		
Control	30.0 ± 12.0	99.5 ± 0.5
$AgNO_3$	2.5 ± 1.0	22.0 ± 3.5
Treatment B		
Dark		
Control	98.5 ± 1.0	
$AgNO_3$	98.0 ± 1.0	
Light		
Control	77.5 ± 6.5	100.0
$AgNO_3$	73.0 ± 5.5	77.0 ± 6.0

apparently of importance for the action of Ag^+ as an Ethrel antagonist, at least with regard to abscission. Paradoxically, cuttings previously sprayed with Ag^+ and placed in the dark without Ethrel treatment abscise more rapidly than do control cuttings. Ag^+ has been found to stimulate ethylene production (1, 10), which might account for enhancement of dark abscission. Abscission stimulation may have been the result of enhanced ethylene production, because the addition of malformin leads to a synergistic action (Table I). The paradox might be explained by considering the differences in time for abscission. When treated with Ethrel in the dark, abscission occurs rapidly, perhaps before loss of Ag^+ activity. On the other hand, stimulation of dark abscission by Ag^+ on cuttings not treated with Ethrel is not evident until 3 days after placement in the dark. The Ag^+ receptor responsible for inhibiting ethylene action may have disappeared by this time. If this hypothesis is true, Ag^+ is probably unsuitable for estimating the role of ethylene in dark abscission or in FR-enhanced abscission (Fig. 3).

The light requirement for inhibition by Ag^+ was demonstrated in various ways. Cuttings sprayed with Ag^+ and incubated in light for 24 h before Ethrel treatment lost much, but not all, of the ability of Ag^+ to negate ethylene action in the dark (Table II). FR irradiation prior to dark incubation resulted in further loss. When cuttings were dark aged prior to treatment with Ag^+ and maintained in the dark during Ethrel treatment, the loss of Ag^+ activity was complete (Table IV), but good activity was restored in the light. However, restoration in white light was considerably less when dark aging and treatment with Ag^+ was preceded by irradiation with FR. These results suggest a role for phytochrome in the formation, activity, or stability of the Ag^+ receptor. Participation of phytochrome in the abscission process was demonstrated earlier (8).

Ethylene stimulates leaf abscission in both light and dark, yet Ag^+ is an effective inhibitor only in the light. This suggests that (a) the receptor for ethylene and Ag^+ are not identical or (b) more than one ethylene receptor is involved, one sensitive to Ag^+ in the light and the other of unknown sensitivity in the light but insensitive to Ag^+ in the dark. Although a light requirement for the antiethylene effect of Ag^+ with regard to leaf abscission is evident, the requirement for light is not universal, because Ag^+ was an effective antagonist on etiolated peas maintained exclusively in the dark (4). An evaluation of light quality and Ag^+ as an ethylene antagonist should be helpful in defining the requirement for light.

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