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Antiethylene Properties of $AgNO_3$ and 2,5-Norbornadiene in Light and Dark in *Vigna radiata*

Roy W. Curtis

Department of Botany and Plant Pathology, Purdue University, W. Lafayette, IN 47907, USA

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Abstract: The ability of the silver ion to prevent ethylene-induced leaf abscission was lost in the dark in both whole seedlings and rootless, bladed explants of *Vigna radiata* when either ethylene or ethephon (an ethylene-releasing compound) were used as defoliating agents. Loss of silver activity in the dark was also observed in bladed explants of *Phaseolus vulgaris* and *Glycine max*. The silver ion was active in the dark in preventing ethylene-induced root curvatures and inhibition of root growth. The antiethylene properties of 2,5-norbornadiene were identical in both the light and dark, undiminished in the presence of ABA, but completely negated by malformin. Because malformin is known to react with sulfhydryl groups, the norbornadiene- and/or ethylene-binding site may contain a sulfhydryl group.

The silver ion inhibits a variety of physiological responses mediated by ethylene, including the "triple response", abscission, senescence (Aharoni et al., 1979, Beyer 1976, 1979, Damilla and VanStaden 1980, Saltveit et al. 1978, Veen and Van de Geijn 1978), rolling of flower rib segments (Beutelmann and Kende 1977), cytokinin accumulation in reproductive organs (VanStaden and Dimalla 1980), and destruction of chloroplast membranes (Saltveit et al. 1978). In *Vigna*, $AgNO_3$ inhibited ethephon-induced leaf abscission from bladed explants in the light but lost considerable activity in the dark and was completely inactive on bladed explants which had been incubated in the dark and treated with ethephon in the dark (Curtis 1981a). Much of the antiethylene activity of the silver ion was recovered when dark-treated explants were returned to the light *prior* to treating with ethephon (Curtis 1981a). The light requirement for

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the antiethylene action of $AgNO_3$ was shown to involve participation of the active form of phytochrome (Curtis 1982a). Later it was shown that the antiethylene properties of silver thiosulphate were also lost in the dark, indicating that the ionic form of silver was not a factor in the activity loss (Curtis 1984a). When the leaf blades were removed from explants of *Vigna* which had been previously sprayed with $AgNO_3$, abscission of the petioles was inhibited in the dark compared to untreated controls (Curtis 1982b). Thus, the antiethylene properties of $AgNO_3$ appeared to be restored in the dark by removing the leaf blades. It was proposed that the phytochrome requirement for the antiethylene activity of Ag^+ on ethylene-induced leaf abscission involved prevention of the formation, accumulation, or transport of a substance in leaves in the dark which negates Ag^+ activity. In these studies it was shown that ABA completely abolished the ability of Ag^+ to inhibit ethylene-induced leaf abscission in the light, and almost completely abolished Ag^+-induced inhibition of petiole abscission from explants in the dark. Subsequently, it was found that methyl jasmonate and ABA-methyl ester diminished or completely negated the antiethylene properties of Ag^+ (Curtis 1984b). The plant growth regulator, malformin, was also shown to negate the antiethylene properties of $AgNO_3$ (Curtis 1981b).

Loss of the antiethylene properties of the silver ion in the dark has not been noted in other studies. For example, $AgNO_3$ was effective in the dark in preventing the triple response induced by ethylene (Beyer 1976). Loss of Ag^+ activity in the dark has been reported in only one species (*V. radiata*), for one physiological response to ethylene (abscission), in rootless explants, and using an ethylene-releasing compound (ethephon) as the source of ethylene. For these reasons, studies are reported here which provide additional information on the loss of Ag^+ activity in the dark. In addition, the antiethylene properties of Ag^+ are compared with those of 2,5-norbornadiene (NDE), an antiethylene compound which inhibits ethylene-induced increases in respiration in tobacco leaves (Sisler and Pian 1973), delays senescence in carnation flowers (Sisler et al. 1983), prevents ethylene action in etiolated pea seedlings (Sisler and Yang 1984), blocks ethylene-induced abscission in citrus leaf explants (Sisler et al. 1985), and blocks ^{14}C-ethylene binding to a Triton X-100 extract from mung beans (Sisler 1982).

Materials and Methods

Loss of Ag^+ Activity in Whole Seedlings of Vigna *in the Dark*

Seedlings of *Vigna radiata* L. Wilczek cv Jumbo were grown in plastic cartons containing vermiculite in a greenhouse under natural light for 14 days, incubated in continuous white fluorescent light (13.5 Wm^{-2}, Champion F90T17/w) or dark (28°C) for 2 days, sprayed to run-off with Tween 20 (0.1% v/v) with or without $AgNO_3$ (1.0 mM), incubated an additional 24 h in the light or dark, and placed in pyrex dishes containing ethephon (1.38 mM, 2-chloroethylphosphonic acid) to a depth of approximately 2 cm. Leaf abscission was determined

at daily intervals by counting the number of leaves which abscised when a 10-g weight was placed briefly on the distal end of the petiole. Experiments in the dark were evaluted under a dim green safe-light.

Loss of Antiethylene Properties of Ag^+ in Rootless, Bladed Explants of Vigna

Bladed explants of *Vigna* in the primary leaf stage (14 days old), obtained by excising 8–9 cm below the apical bud, were placed in 250 ml beakers (20/beaker) containing deionized H_2O, incubated and sprayed in the light or dark as described above, transferred to 10-1 desiccators, sealed, and treated with ethylene gas (100 μl/l). Leaf abscission in the dark was determined 24 h after treatment with ethylene and 48 h after treatment in the light.

Loss of Ag^+ Activity in Other Species in the Dark

Rootless, bladed explants of *Glycine max* Merr cv Amsoy 70 (19 days old, growth above primary leaves removed) and *Phaseolus vulgaris* L cv Harvester (14 days old) were placed in beakers containing H_2O, incubated in the light or dark, sprayed as described, and transferred to solutions of ethephon (2.76 mM). Leaf abscission was determined at daily intervals as described.

Antiethylene Properties of 2,5-*Norbornadiene (NDE) in the Light and Dark*

Bladed explants of *Vigna* were transferred to beakers containing H_2O and incubated for 3 days in the light or dark, transferred to desiccators, sealed, and treated with ethylene (100 μl/l) and various concentrations of NDE (expressed as liquid NDE/l air). Thus, 12 μl of liquid NDE/l of air is equivalent to 2700 μl gaseous NDE/l air. Abscission in the dark was determined after 24 h and after 48 h in the light.

Effect of $AgNO_3$ and NDE on Petiole Abscission from Bladeless Explants of Vigna

Bladeless explants consisted of two opposite petioles (~2 mm long) subtended by a stem (~8 mm long) and were prepared by excising the primary leaf blades from the distal ends of the petioles and the stem 8 mm below the junction of the petioles and the stem. For $AgNO_3$ treatment the explants were inserted stem end down (proximal treatment) or petiole ends down (distal treatment) into aluminum planchets (3.1 cm O.D., 0.2 cm deep, 20 explants/planchet) containing 1.2 ml 1% (w/v) water agar containing $AgNO_3$. Planchets were placed in pyrex baking dishes (2.7 l) lined with moist paper towels, covered with aluminum foil and placed in the dark. For NDE treatment the petioles were placed

placed in the dark (28°C). Petiole absission was determined at various intervals by counting the number of petioles that abscised when subjected to a 5.0-g weight placed briefly on the end of the petiole.

Effect of ABA and Malformin on Antiethylene Properties of 2,5-Norbornadiene

Bladed explants were placed in beakers (125-ml, 20/beaker) containing 50 ml ABA (10^{-4} M) or malformin (10^{-5} M), incubated for 24 h in continuous white light, transferred to desiccators, sealed, treated with ethylene or ethylene *plus* NDE (25 μl/l). Controls were placed in beakers containing water and treated similarly with ethylene in the presence or absence of NDE.

ABA was also tested for effects on the biological activity of NDE on bladeless explants. The bladeless expants were inserted petiole ends down (distal treatment) into planchets containing water agar with or without ABA (10^{-4}–10^{-6} M), and incubated in the dark in desiccators with or without NDE (12 μl/l). Abscission was determined after 48 h.

Effect of $AgNO_3$ and 2,5-Norbornadiene on Corn Root Growth and Ethylene-Induced Root Curvatures in the Dark

Corn seeds (*Zea mays* L WF9X38-11) were washed and placed in petri dishes on the periphery of Whatman No. 1 filter paper (9.0 cm) previously moistened with 4.0 ml water or 1.0 mM $AgNO_3$. The seeds (10/dish) were arranged in sets of five on opposite sides of the dish so that the roots would grow toward one another, and incubated in desiccators containing air or ethylene (100 μl/l) for 72 h in the dark (28°C). Root curvatures were determined by counting the number of roots with at least a 90° curvature from the original direction of growth. Fifty seeds were used for each treatment in each of two experiments.

The antiethylene properties of NDE were tested by germinating seeds on filter paper moistened with water or solutions of ethephon (69, 690 μM) in sealed desiccators containing air or NDE (25 μl/l).

Results

Antiethylene Properties of $AgNO_3$ in Whole Seedlings of Vigna *in Light and Dark*

The antiethylene properties of $AgNO_3$ in whole seedlings of *Vigna* after incubating for two days in continuous light or in the dark were examined with ethephon as the source of ethylene. In the light, $AgNO_3$ completely inhibited leaf abscission for two days after the addition of ethephon and inhibited strongly up to four days (Table 1). In the dark, however, $AgNO_3$ was completely inactive in preventing ethephon-induced leaf abscission.

Table 1. Loss of the antiethylene properties of $AgNO_3$ in whole seedlings of *Vigna* in the dark.

Treatment[a]	Abscission (%)			
	Day 1	Day 2	Day 3	Day 4
In light				
Control	0	23.1	29.5	30.8
$AgNO_3$	0	0	2.1	13.5
In dark				
Control	85.1	100	—	—
$AgNO_3$	94.0	100	—	—

[a] Whole seedlings of *Vigna* incubated for 2 days in continuous white light or in the dark, sprayed with Tween 20 (0.1%) with or without $AgNO_3$ (1.0 mM), incubated an additional day in the light or dark, and placed ~2 cm deep in pyrex dishes containing ethephon (1.38 mM). Leaf abscission determined daily after ethephon treatment. Average 2 determinations. No abscission occurred on either controls or silver-treated seedlings in the absence of ethephon in either light or dark.

Table 2. Failure of $AgNO_3$ to inhibit ethylene- or ethephon-induced leaf abscission from bladed explants of *Vigna* in the dark.

Treatment[a]	Abscission (%)
Ethylene treated	
In light	
Control	71.2 ± 8.7
$AgNO_3$	5.0 ± 0.1
In dark	
Control	92.5 ± 2.2
$AgNO_3$	92.5 ± 2.0
Ethephon treated (dark only)	
Control	51.3 ± 7.7
$AgNO_3$	79.5 ± 6.5

[a] Bladed explants placed in beakers containing water, incubated for 2 days in continuous white light or in the dark, sprayed with Tween 20 (0.1%) with or without $AgNO_3$ (1.0 mM), incubated an additional 24 h in the light or dark, transferred to desiccators, and treated with ethylene (100 μl/l) or transferred to beakers containing ethephon (1.38 mM). In the absence of ethylene or ethephon, abscission of controls and silver-treated explants in the dark was 18.5 and 30.5%, respectively. Leaf abscission determined after 24 h in the dark or after 48 h in the light. Results are means ± SE.

Loss of Antiethylene Properties of $AgNO_3$ in Bladed Explants of Vigna *Treated with Ethylene*

Bladed explants were incubated and treated with Tween 20 with or without $AgNO_3$ as described, transferred to desiccators, and treated with ethylene in the light or dark. For comparison, other bladed explants were treated with ethephon solutions in open beakers. In the light, $AgNO_3$ inhibited ethylene-induced abscission strongly but was completely inactive in the dark (Table 2). When ethephon was used as an ethylene source in the dark, $AgNO_3$ was not only completely inactive in preventing abscission but appeared to enhance abscission rates.

Table 3. Loss of the antiethylene properties of $AgNO_3$ in the dark in bladed explants of *Glycine* and *Phaseolus*.

Treatment[a]	Abscission (%) Day 1	Day 2	Day 3
Glycine max			
In light			
Control	0	16.4 ± 1.5	79.4 ± 2.5
$AgNO_3$	0	0.9 ± 0.9	16.4 ± 1.9
In dark			
Control	0	71.5 ± 16.4	—
$AgNO_3$	63.8 ± 13.3	90.3 ± 3.7	—
Phaseolus vulgaris			
In light			
Control	1.2 ± 0.7	98.4 ± 1.5	100
$AgNO_3$	1.2 ± 0.8	34.3 ± 7.2	64.4 ± 8.6
In dark			
Control	24.0 ± 7.3	94.8 ± 2.5	96.1 ± 3.8
$AgNO_3$	26.5 ± 11.5	69.6 ± 7.6	84.6 ± 0.2

[a] Bladed explants placed in beakers containing water, aged for 2 days in continuous white light or in the dark, sprayed with Tween 20 (0.1%) with or without $AgNO_3$ (1.0 mM), aged an additional day in light or dark, and transferred to solutions of ethephon (2.76 mM). Abscission determined at daily intervals after ethephon treatment. Results are ±SE. No abscission occurred from *Glycine* or *Phaseolus* in the absence of ethephon in light or dark.

Loss of Antiethylene Properties of $AgNO_3$ in Glycine *and* Phaseolus *in the Dark*

Bladed explants of *G. max* and *P. vulgaris* were used to determine if $AgNO_3$ activity is lost in the dark in species other than *Vigna*. In the light, $AgNO_3$ inhibited ethephon-induced leaf abscission in both *Glycine* and *Phaseolus* (Table 3). In the dark, $AgNO_3$ was not only completely inactive in inhibiting ethephon-induced leaf abscission in *Glycine* but appeared to stimulate abscission itself. The antiethylene properties of $AgNO_3$ in *Phaseolus* were diminished considerably in the dark compared to activity in light.

Antiethylene Properties of Norbornadiene in the Light and Dark

Bladed explants, incubated for 3 days in the light or dark, were used to determine the antiethylene properties of NDE (Fig. 1). As the concentration of NDE was gradually increased from 3.1 to 25 μl/l, the ability of ethylene to induce leaf abscission gradually diminished in both light and dark. No significant difference was observed in NDE activity in light or dark. At 50 μl/l, NDE completely inhibited ethylene action, but NDE also appeared to be toxic at this concentration (petioles turned brown, felt soft).

Effect of NDE and $AgNO_3$ on Petiole Abscission from Bladeless Explants of Vigna

The effect of NDE on petiole abscission from bladeless explants was determined in the presence and absence of ethylene. In the absence of ethylene,

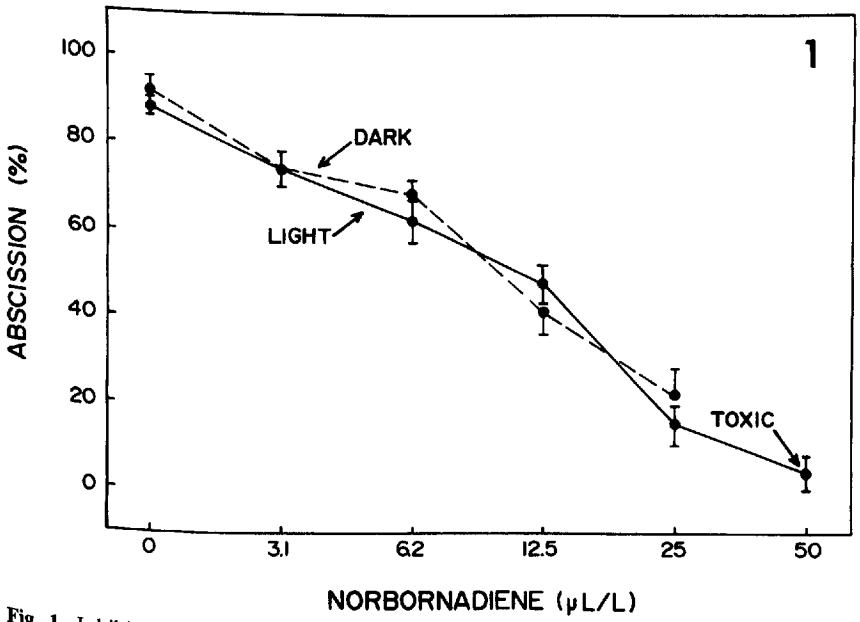

Fig. 1. Inhibition of ethylene-induced leaf abscission by 2,5-norbornadiene in *Vigna* explants in the light and dark. Bladed explants placed in beakers containing water, incubated for 3 days in continuous white light or in the dark, transferred to desiccators, and treated with ethylene (100 μl/l) and various concentrations of norbornadiene. Leaf abscission determined after 24 h (dark) or 48 h (light). Vertical bars are ±SE. In the absence of ethylene, abscission in light and dark was 0 and 15.3%, respectively.

NDE inhibited petiole abscission about 60% at 12.5 μl/l, and completely at 25 μl/l (Fig. 2). Although substantial NDE activity was lost in the presence of ethylene, significant inhibition of abscission remained.

When applied distally, $AgNO_3$ also inhibited petiole abscission from bladeless explants (Fig. 3), but the degree of inhibition appeared to be less than when the bladeless explants were obtained from bladed explants which had been sprayed with $AgNO_3$ and incubated for 24 h in the light prior to deblading (Curtis 1982b, 1984b). When applied proximally, $AgNO_3$ at higher concentrations stimulated petiole abscission slightly after 24 h, but by 48 h had no significant effect on petiole abscission. At no concentration did proximally-applied $AgNO_3$ inhibit petiole abscission significantly. Similar results were obtained when proximally- and distally-applied $AgNO_3$ was applied in continuous white light (results not given).

When solutions of $AgNO_3$ were mixed with agar, the mixture turned red to pink, depending on the concentration of $AgNO_3$. It seemed that the apparent diminished activity of distally-applied $AgNO_3$ might have resulted from a lowering of the $AgNO_3$ concentration by reaction with agar. However, when $AgNO_3$ was extracted with cold water from the solidified mixture and examined for antiethylene activity by spraying on bladed explants and treating with ethephon, the activity appeared to be identical with that of untreated $AgNO_3$ (results not given). If the concentration of $AgNO_3$ was diminished by incorporation into water–agar, the decrease was too small to be detected.

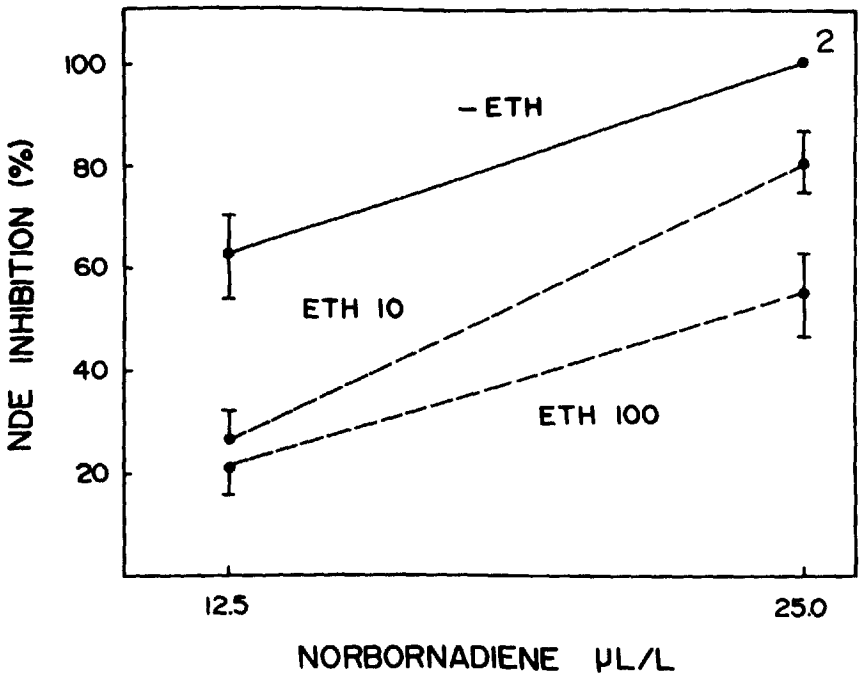

Fig. 2. Effect of 2,5-norbornadiene on petiole abscission from bladeless *Vigna* explants in the presence or absence of ethylene. Bladeless explants inserted petiole ends down into water–agar, placed in desiccators, treated with various concentrations of norbornadiene in the presence or absence of ethylene, and placed in the dark. Abscission determined after 48 h. Vertical bars are ± SE. NDE inhibition based on abscission rate (76.7 ± 3.0%) of similar explants in the absence of NDE and ethylene.

The ability of ethylene to reverse the antiethylene properties of $AgNO_3$ was also examined. Bladeless explants were prepared from bladed explants which had been sprayed with or without $AgNO_3$ and maintained for 24 h in the light. The bladeless explants were incubated in desiccators with or without ethylene (Table 4). In the absence of ethylene, $AgNO_3$ inhibited petiole abscission, but the inhibition was almost completely negated by the addition of ethylene even at concentrations as low as 1 µl/l.

Effect of ABA and Malformin on Antiethylene Properties of NDE

Pretreatment of bladed explants with ABA had no significant effect on the antiethylene properties of NDE compared with controls treated with water (Fig. 4). The antiethylene properties of NDE, however, were completely negated in bladed explants which had been previously treated with malformin even when the ethylene concentration was only 1 µl/l. Neither ABA nor malformin induced substantial abscission when the bladed explants were incubated in the light in desiccators to which ethylene was not added.

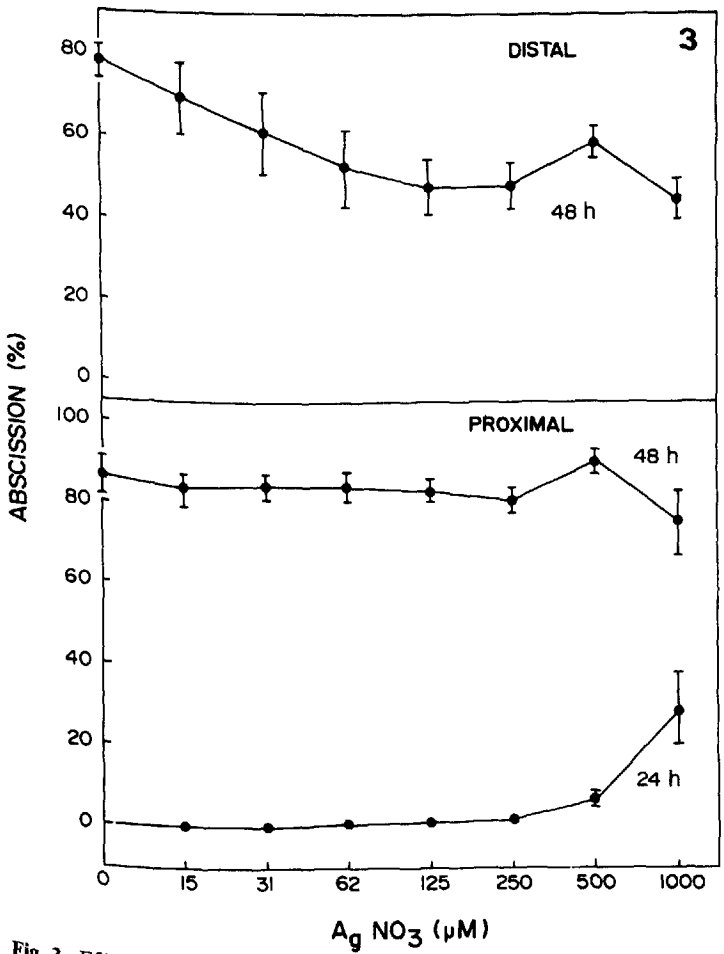

Fig. 3. Effect of $AgNO_3$ on petiole abscission from bladeless explants of *Vigna*. Bladeless explants inserted petiole ends down (distal treatment) or stem end down (proximal treatment) into water–agar with or without $AgNO_3$, placed in pyrex baking dishes lined with moist paper towels, covered with aluminum foil and placed in the dark. Petiole abscission determined after 48 h (distal treatment) or 24 and 48 h (proximal treatment). Vertical bars are ± SE.

In bladeless explants, ABA appeared to negate slightly the pronounced inhibition of abscission induced by NDE (Table 5). Maximum negation by ABA (10^{-5} M) was approximately equal to the amount by which ABA stimulated petiole abscission in the absence of NDE.

Antiethylene Properties of $AgNO_3$ and NDE in Corn Roots in the Dark

When corn seeds were germinated in the presence of ethephon, roots were severely curved and fresh weight was diminished (Fig. 5). In the presence of

Table 4. Negation of antiethylene properties of $AgNO_3$ in bladeless explants of *Vigna* by ethylene.

Treatment[a]	Abscission (%)
Air	
Control	70.8 ± 4.0
$AgNO_3$	10.4 ± 4.7
Ethylene (100 μl/l)	
Control	97.5 ± 1.2
$AgNO_3$	83.7 ± 5.0
Ethylene (10 μl/l)	
Control	99.2 ± 0.8
$AgNO_3$	88.3 ± 6.0
Ethylene (1 μl/l)	
Control	98.3 ± 1.7
$AgNO_3$	88.3 ± 5.6

[a] Bladeless explants prepared from bladed explants which had been sprayed 24 h earlier with Tween 20 (0.1%) with or without $AgNO_3$ (1.0 mM) and maintained in continuous white light. Bladeless explants inserted petiole ends down into water agar and placed in desiccators with or without ethylene. Abscission determined after 48 h in the dark.

NDE, both of these effects of ethephon were completely prevented. When solutions of $AgNO_3$ were mixed with ethephon solutions, a white precipitate resulted. Consequently, ethylene itself was used to examine the antiethylene properties of $AgNO_3$ in the dark (Table 6). $AgNO_3$ reduced by more than 50% the number of roots which curved in response to ethylene. In experiments with ethephon, both corn and *Vigna* seeds were germinated for 2 days on filter paper moistened with water or $AgNO_3$ (1.0 mM) and transferred to filter paper moistened with ethephon (0.14 mM). After 2 days, $AgNO_3$ completely prevented (*Vigna*) or alleviated (corn) inhibition of root fresh weight increment induced by ethephon (unpublished observations).

Discussion

Dark loss of the antiethylene properties of the silver ion in previous studies employed only rootless, bladed explants of *Vigna* and ethephon, an ethylene-releasing compound, as the source of ethylene (Curtis 1981a, 1984a). However, the loss in activity appears to be a general phenomenon unrelated to limitations in the earlier experiments. For example, the presence or absence of roots had no effect on the loss in activity of the silver ion, because the silver ion was completely inactive in preventing ethephon-induced leaf abscission in the dark on whole seedlings of *Vigna* (Table 1). Nor is the loss in activity peculiar to *Vigna*, because the antiethylene properties of the silver ion were considerably diminished in preventing ethephon-induced leaf abscission in *P. vulgaris* in the dark, and completely lost in *G. max* (Table 3). In *G. max*, $AgNO_3$ even appeared to enhance ethephon-induced leaf abscission in the dark. In both *P. vulgaris* and *G. max*, the silver ion effectively inhibited ethephon-induced leaf abscission in the light.

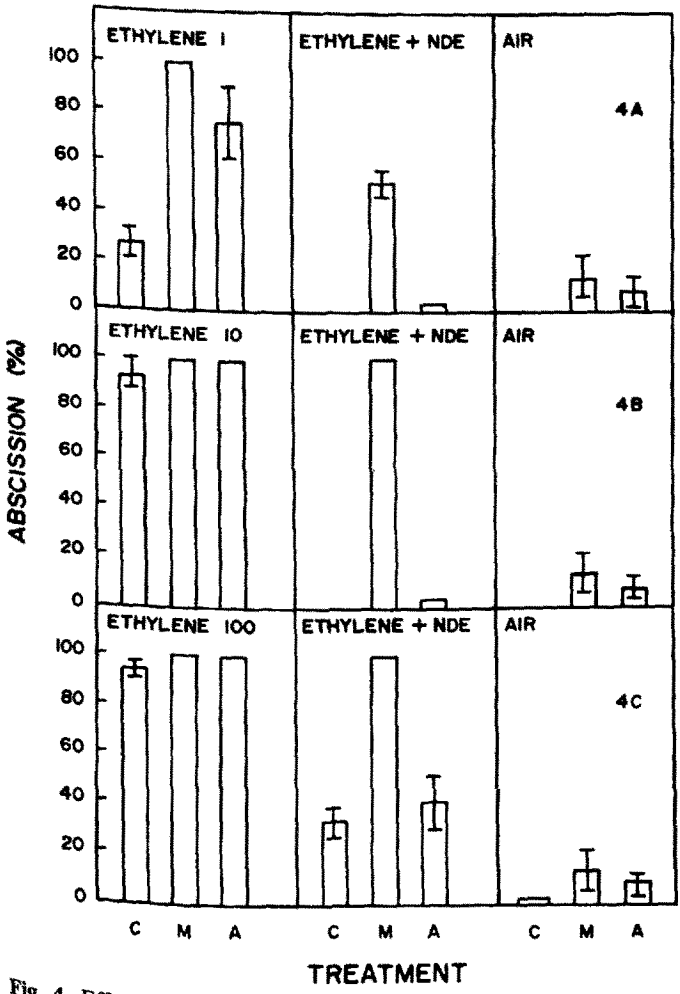

Fig. 4. Effect of ABA and malformin on antiethylene properties of 2,5-norbornadiene. Bladed explants placed in beakers containing H_2O (C), ABA (A) 10^{-4} M or malformin (M) 10^{-5} M, incubated for 24 h in continuous light, transferred to desiccators, and treated with ethylene or ethylene *plus* norbornadiene (25 μl/l). Neither ethylene nor norbornadiene added to control (air) desiccators. Leaf abscission determined after 48 h in continuous white light. Vertical bars are ±SE.

From early work on the use of ethephon as a defoliating agent it was concluded that the stimulation of abscission by ethephon was mediated only by ethylene (Morgan 1969). This conclusion was questioned by others (Zubkova and Markina 1977) who observed that the defoliating activity of ethephon was considerably greater at pH 2.23 than at pH 4.13, but that ethylene released from the treated leaves was greater at the higher pH. For this reason the loss of the antiethylene properties of the silver ion in the dark was examined using ethylene itself (Table 2). The silver ion was completely inactive in the dark in preventing either ethylene- or ethephon-induced leaf abscission, and the loss

Table 5. Effect of ABA on inhibition of petiole abscission from bladeless explants of *Vigna* by 2,5-norbornadiene.

Treatment[a]	Abscission (%)
Air	
Control	73.6 ± 3.3
ABA 10^{-6} M	96.6 ± 1.6
ABA 10^{-5} M	98.3 ± 1.6
ABA 10^{-4} M	78.9 ± 9.0
NDE (12 μl/l)	
Control	9.6 ± 3.9
ABA 10^{-6} M	8.2 ± 6.0
ABA 10^{-5} M	30.6 ± 6.6
ABA 10^{-4} M	22.8 ± 7.8

[a] Bladeless explants inserted petiole ends down into water–agar with or without (control) ABA and incubated for 48 h in desiccators with or without NDE in the dark.

cannot be attributed to the use of ethephon. The ability of the silver ion to prevent ethephon-induced leaf abscission in the light (Curtis 1981a) and of NDE to completely prevent ethephon-induced root curvatures and inhibition of root growth (Fig. 5) support the proposal that ethylene-like responses following ethephon treatment are mediated by ethylene (Morgan 1969).

Although antiethylene properties of the silver ion in the dark have not been extensively examined, loss of these properties in the dark appear to be restricted to ethylene-induced leaf abscission, and removal of the leaf blades in the dark restores the antiethylene properties of the silver ion (Curtis 1982b). The silver ion was also active in the dark in preventing the triple response induced by ethylene (Beyer 1976) and in preventing ethylene-induced epinastic responses in *Euphorbia pulcherrina* Willd. in the dark (Reid et al. 1981). In the present studies, the silver ion inhibited ethylene-induced root curvatures in corn in the dark (Table 6) and in *Vigna* and corn, completely prevented inhibition of root fresh weight caused by ethephon treatment in the dark (unpublished observations).

Both the silver ion and NDE were active in inhibiting ethylene-induced abscission from bladed explants in the light, but unlike the silver ion, the activity of NDE was virtually identical in the dark (Table 1, Fig. 1). Differences between the activity of the silver ion and NDE were also observed in bladeless explants of *Vigna*. Both compounds inhibited petiole abscission in the absence of exogenous ethylene, but their activity differed in the presence of ethylene. Although considerable NDE activity was lost in the presence of ethylene (10 and 100 μl/l, Fig. 2), inhibition by NDE was still significant. In contrast, the ability of the silver ion to inhibit petiole abscission was completely negated after the same period even at concentrations of ethylene as low as 1 μl/l (Table 4). These differences might be explained by the methods used to assess the effects of ethylene on the antiethylene properties of the two compounds. In the case of NDE, relatively high concentrations of NDE were continually present in the desiccator to inhibit ethylene action on the debladed explants. In the case of $AgNO_3$, however, only silver ions present in the debladed explants at

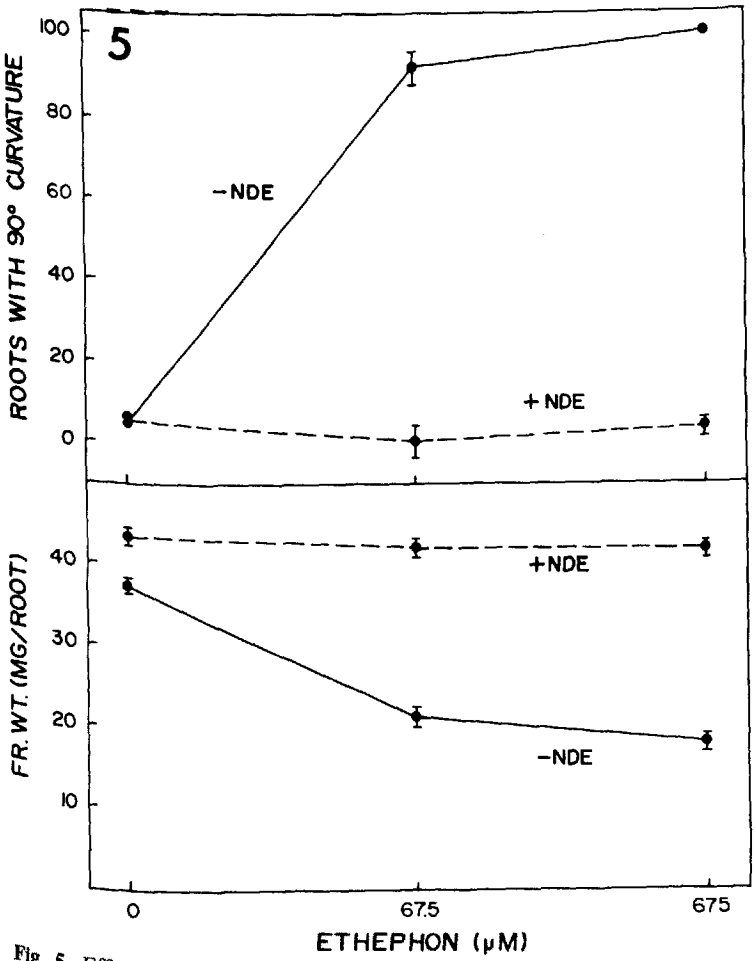

Fig. 5. Effect of 2,5-norbornadiene on ethephon-induced root curvatures and inhibition of root growth. Corn seeds germinated for 3 days on filter paper moistened with H_2O or ethephon solutions in sealed desiccators with or without norbornadiene (25 μl/l). Root curvatures determined by counting the number of roots with at least a 90° curvature from the original direction of growth (100 roots/treatment). Primary roots excised and weighed. Results are ±SE.

the beginning of the experiment were available to block the action of subsequently added ethylene. In any case, it is apparent that the inhibition of petiole abscission from bladeless explants by $AgNO_3$ is readily negated by ethylene whereas bladed explants treated with $AgNO_3$ in the light respond but little to ethylene.

Major differences in the effect of ABA and malformin on the antiethylene properties of the silver ion and NDE in bladed explants were found. In *Vigna*, both ABA and malformin completely negated the antiethylene properties of the silver ion (Curtis 1981b, 1982b, 1984b), but only malformin negated the antiethylene properties of NDE in bladed explants even at the lowest concen-

Table 6. Effect of $AgNO_3$ on maize root curvatures induced by ethylene in the dark.

Treatment[a]	Roots curved (%)
Air	
Control	2.0 ± 1.3
$AgNO_3$	0
Ethylene	
Control	68.0 ± 6.6
$AgNO_3$	29.0 ± 6.2

[a] Maize seeds germinated for 3 days on filter paper moistened with H_2O (control) or solutions of $AgNO_3$ (1.0 mM) in desiccators in the presence or absence of ethylene (100 μl/l). Root curvatures determined by counting the number of roots with a 90° or greater curvature from the original direction of growth. Results are means ± SE.

tration of ethylene (1 μl/l, Fig. 4a–c). Since both malformin and ABA increase the sensitivity of *Vigna* to ethylene-induced leaf abscission (Curtis 1971, 1984b), yet only malformin negates NDE, it seems likely that the mode of action of malformin and ABA are different and that potentiation of ethylene action alone cannot explain the ability of malformin to negate NDE action.

In the absence of exogenous ethylene, ABA did negate slightly the inhibition by NDE of petiole abscission from bladeless explants (Table 5). This was in marked contrast to the complete negation by ABA of the inhibition of petiole abscission by the silver ion in similar bladeless explants (Curtis 1984b). Malformin failed to negate NDE activity in bladeless explants (unpublished observations) and also failed to negate $AgNO_3$ activity in similar experiments (Curtis 1982b).

The reason for the loss of the antiethylene properties of the silver ion in the dark is unknown. Since removal of the leaf blade restores the ability of the silver ion to inhibit petiole abscission in the dark, it seems reasonable to postulate that a substance originating in the leaf blade negated action by the silver ion (Curtis 1982b). From the present work, indirect evidence suggests that the substance could be ABA. For example, the antiethylene properties of NDE are stable in the dark (Fig. 1) and in the presence of ABA (Fig. 4). The silver ion, however, is inactive in the dark and also negated by ABA. Although methyl jasmonate also negates the antiethylene properties of the silver ion, the negation was slight compared to the complete loss in activity of the silver ion in the dark (Curtis 1984b).

Because malformin negates the antiethylene properties of the silver ion (Curtis 1981b), is believed to bind to sulfhydryl groups in higher plants (Suda and Curtis 1964, Ciarlante and Curtis 1977), and is known to react with sulfhydryl-containing compounds (Iriuchijima and Curtis 1970), it was suggested that the ethylene and/or silver binding site contains a sulfhydryl group (Curtis 1981b). The ability of malformin to negate the antiethylene properties of NDE (Fig. 4) also suggests that the NDE- and/or ethylene-binding site contains a sulfhydryl group. Based on the inhibition of ethylene binding to a Triton X-100 extract of mung beans by sulfhydryl reactants, the presence of a sulfhydryl group at or near the active ethylene-binding site has been suggested elsewhere (Sisler 1982). Since NDE has not been reported to react with sulfhydryl groups

it is unlikely that malformin competes with NDE for binding sites, but it may interfere with NDE binding in other ways. In some ethylene mediated processes (stem swelling, inhibition of hook opening, inhibition of stem elongation) it is possible that malformin also inhibits ethylene binding, because malformin inhibits each of these ethylene activities (Curtis 1971). With regard to abscission processes, however, malformin has the opposite effect of potentiating ethylene-induced abscission, as well as negating the antiethylene properties of the silver ion and NDE, compounds which are believed to interfere with ethylene action by attaching at or near the ethylene-binding site. Solution of this paradox would be of assistance in studies on ethylene action, abscission processes, and malformin action.

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