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Anatomical and Physiological Effects of Silver Thiosulfate on Ethylene-Induced Abscission in *Coleus*

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Abstract. Petioles of explants of *Coleus blumei* Benth. exposed to 20 μl/l ethylene abscised within 36 h. Pretreatment of explants with 4 mM silver thiosulfate (STS) inhibited ethylene-induced abscission. Delaying treatment with STS reduced its effectiveness in retarding ethylene-promoted abscission, suggesting that some events leading to abscission are initiated during the first hours of ethylene treatment. Microscopic study of abscission zones of ethylene-treated explants showed greatly increased amounts of rough endoplasmic reticulum, disruptions of the plasma membrane, and some cell separation in the region of the middle lamella. Pretreatment with STS prevented ethylene-induced reorganization of the endomembrane system and the subsequent middle lamellar dissolution.

The correlation between ethylene and the promotion of fruit, floral, or foliar abscission has been recognized for over 80 years (Neljubow 1901, Abeles 1973). Elevated levels of ethylene in plar tissues may be the result of natural aging processes (maturation), environmental stress, invasion of pathogens, or the application of ethylene-inducing chemicals (Addicott 1970, 1982, Abeles 1973).

Beyer (1976) reported that foliar application of silver nitrate ($AgNO_3$) effectively blocked ethylene-induced abscission in leaves, flowers, and fruits. Subsequent studies have supported this work (Greene 1980, Miranda and Carlson 1981), and investigators have used the $AgNO_3$ system as a probe for the site(s) of ethylene and/or Ag^+ binding (Curtis 1981, 1982). However, the relative immobility of $AgNO_3$ within plant tissue (Veen and Van de Geijn 1978) as well as some phytotoxic effects of $AgNO_3$ at effective application levels (Cameron and Reid 1981) have limited the commercial usefulness of this compound as an antiethylene agent.

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Silver thiosulfate (STS; $Ag_2S_2O_3$) is highly mobile within the plant (Veen and Van de Geijn 1978) and possesses antiethylene properties similar to those described for $AgNO_3$ (Veen and Van de Geijn 1978, Reid et al. 1980, Mor et al. 1981). Little is known about the site of Ag^+ binding in the plant (Curtis 1981, 1982) or the physiological and anatomical characteristics of silver inhibition of ethylene effects on plants (Curtis 1981, 1982, Valdovinos et al. 1981). This study was initiated to investigate some of the physiological and anatomical changes associated with STS and ethylene treatment in the *Coleus* explant abscission system.

Materials and Methods

Plant Materials

Coleus cuttings (*Coleus blumei* Benth.) were grown in a greenhouse with a minimum night temperature of 20°C. Established plants were pinched to encourage branching. Explants from these branches consisted of the petioles and node of the second pair of fully expanded leaves and part of the internode above and below. Prior to deblading, basal ends of one group of explants were placed in a 4-mM solution of STS prepared as described by Reid et al. (1980) for 15 min. All explants were then debladed and trimmed, leaving a 1.0-cm stub of each petiole, 1.0 cm of the distal internode, and 4.0 cm of the proximal internode.

Treatment with Ethylene

In three replicate experiments, explants were placed in small vials of deionized water, then sealed in 1.0-l Mason jars ventilated with flowing streams of air (5 l/h). Air for control treatments was passed through a tower containing potassium permanganate on alumina. Ethylene in air mixtures (20 μl/l) was obtained by diluting ethylene from premixed cylinders into airstreams using flowboards (Cameron and Reid 1981). Ethylene concentrations were checked by gas chromatography. At 12-h intervals the number of abscised petioles was recorded.

Effect of Delayed Application of STS

Explants were placed in a chamber ventilated with air containing 20 μl/l ethylene. To test the effect of delayed STS treatment or removal of the ethylene stimulus on the progress of events in the abscission process, explants were treated with ethylene for 0, 1, 2, or 4 h. Replicates were then treated in one of three different ways: 1) removed from the chamber and placed in ethylene-free air; 2) removed from the chamber, injected into the nodal region through the distal explant surface with 20 μl of 4 mM STS, and placed in ethylene-free air; or 3) removed from the chamber, injected with STS, and then replaced in ethylene. The number of abscised petioles was recorded at regular intervals. Data were obtained from three replicate experiments.

Light Microscopy

For light microscopy (LM), abscission zone samples from STS-ethylene-treated, ethylene-treated, and control (air-treated) explants were collected at regular intervals from 2 to 24 h, fixed in FAA (formalin–acetic acid–alcohol) for 18 h, dehydrated through a graded ethanol series, and embedded in glycol methacrylate plastic (Dupont-Sorvall). Sections were cut at 2-3 μm with a glass knife in a JB-4 microtome, mounted on glass slides, and stained for 30 sec with 0.2% Toluidine blue. Slides were examined and photographed with a Zeiss photomicroscope.

Electron Microscopy

For transmission electron microscopy (TEM), abscission zone samples were taken at regular intervals from 1 to 8 h, fixed for 2 h in 4% glutaraldehyde in 0.1 M cacodylate buffer pH 6.8, rinsed, and postfixed in 1% buffered OsO_4 for 18 h. Samples were then rinsed and dehydrated through a graded ethanol series and embedded in Spurr's (1969) epoxy resin. Sections were cut at 600–900 Å on a diamond knife in a Sorvall MT-2 ultramicrotome, mounted on uncoated copper grids, and stained for 30 min in saturated uranyl acetate solution followed by 30 min in lead citrate (Reynolds 1963). Specimens were examined in a JEOL 100S electron microscope at 80 kV.

Results

Effect of Ethylene and STS on Abscission of Coleus Explants

Preliminary experiments indicated that natural abscission in control (air-treated) explants was initiated after 108 h and was completed 132 h after excision. In explants treated with 20 μl/l ethylene, petioles began to abscise after approximately 18 h (Fig. 1). Abscission was complete in all these explants within 36 h of the start of treatment. Pretreatment of explants with 4 mM STS delayed ethylene-induced abscission: after 72 h 30% of the petioles had abscissed, and abscission was complete in 132 h (Fig. 1).

Effect of Delayed Application of STS

Removal of the ethylene stimulus decreased petiole abscission in all treatments (Fig. 2). Abscission was further inhibited by treatment with STS, although this inhibition became less marked the longer the tissue had been pretreated with ethylene. Least abscission was observed in control petioles treated with STS at T_0.

In explants treated continuously in ethylene, abscission was complete by 36 h (Fig. 1). In these explants, STS pretreatment delayed abscission (Fig. 3).

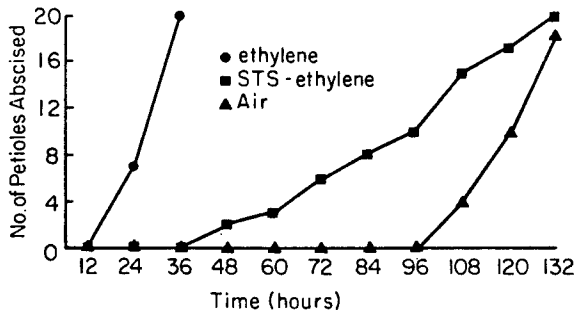


Fig. 1. Effect of different treatments on petiole abscission over time in *Coleus* stem explants. (●) Explants continuously exposed to ethylene; (■) explants pretreated with STS and maintained in ethylene; (▲) untreated explants maintained in air.

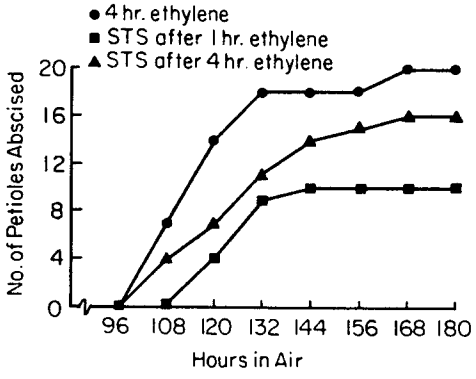


Fig. 2. Effect of STS applied at selected times on petiole abscission for stem explants pretreated with ethylene for 4 h and then removed to air: (●) 4-h ethylene pretreatment, no STS; (■) STS applied 1 h after exposure to ethylene; (▲) STS applied 4 h after exposure to ethylene.

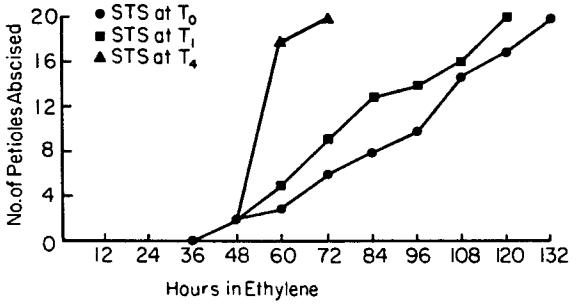
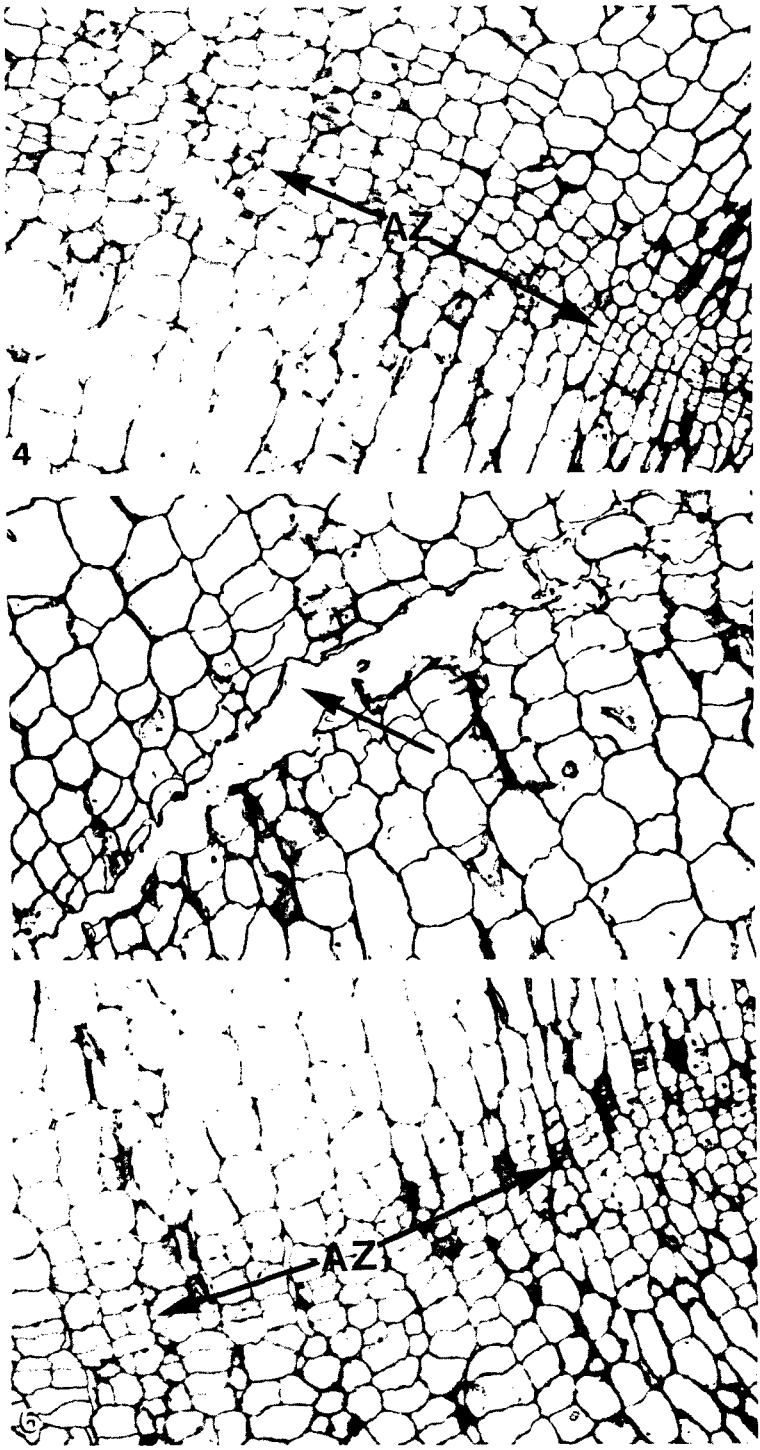


Fig. 3. Effect of STS applied at selected times on petiole abscission for stem explants maintained continuously in ethylene. Data are from a representative experiment of three replicate trials. (●) STS applied at the start of ethylene exposure (T_0); (■) STS applied 1 h after exposure to ethylene (T_1); (▲) STS applied 4 h after exposure to ethylene (T_4).

When STS was injected at intervals after the initiation of ethylene treatment, it was less effective in inhibiting abscission. Treatment with STS after 1 h of ethylene treatment resulted in somewhat less inhibition of abscission than treatment at T_0 (Fig. 3). After 4 h of ethylene treatment, STS was only marginally effective in delaying abscission.

Figs. 4–6. LM of control, ethylene-treated, and STS-ethylene-treated tissue. **Fig. 4.** Untreated control tissue. Abscission zone (AZ) consisting of 4–5 layers of laterally elongate, rectangular cells located at the base of the petiole. ×200. **Fig. 5.** Disruption of cells in the abscission zone resulting in the formation of a separation cavity (arrow) after 12 h of ethylene treatment. ×225. **Fig. 6.** STS-pretreated tissue after 20 h of ethylene treatment. Note that there is little evidence of disruption in the cells of the abscission zone (AZ). ×235.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Microscopic Observations

LM studies of sections from control (air-treated, 0 h) explants showed that the intact *Coleus* abscission zone consisted of 4–5 layers of laterally elongate, rectangular cells located at the base of the petiole (Fig. 4). Cells were highly vacuolate, with peripherally located nuclei. Radial and longitudinal disruption of cells in the abscission zone occurred 6–8 h after the beginning of ethylene treatment. Disruption resulted in the formation of separation cavities which extended from the abaxial side of the petiole to the vasculature. Twelve hours after the onset of ethylene treatment, separation was extensive both abaxially and adaxially, and cell wall debris was often observed in the separation cavities (Fig. 5).

Pretreatment of explants with STS appeared to limit the abscission process for at least 24 h. Nuclei were more conspicuous in STS-treated material than in ethylene controls; however, samples taken after 20 h showed no evidence of cellular disruption (Fig. 6).

TEM examination of control (air-treated, 0 h) abscission zone cells showed that the cytoplasm was homogenous and confined to the periphery of the cell by the large central vacuole (Fig. 7). The nucleus was large and elongate. Organelle and membrane systems were intact; short strands of rough endoplasmic reticulum (RER) were present in the cytoplasm. One hour after commencement of ethylene treatment, profiles of RER, polyribosomes, and energized mitochondria were observed more frequently than in uninduced tissue (Fig. 8). Membrane invaginations, vesiculation, and paramural bodies were characteristic of cells after 2 h of ethylene treatment, and segments of RER were abundant. In some cells the plasma membrane appeared to be separated from the cell wall, and vesicular or fibrillar material was visible between the wall and plasma membrane (Fig. 9). Progressive cytoplasmic degeneration was evident 4–6 h after ethylene treatment. There was no evidence of extensive cell wall separation during this experiment, but areas of disruption between the plasma membrane and cell wall (Fig. 9) and dissolution of the middle lamella were frequently noted.

In contrast to the ethylene-treated control tissue, STS-pretreated tissue showed little ultrastructural change as a result of ethylene treatment. Samples taken after 6 h of ethylene treatment were essentially indistinguishable from

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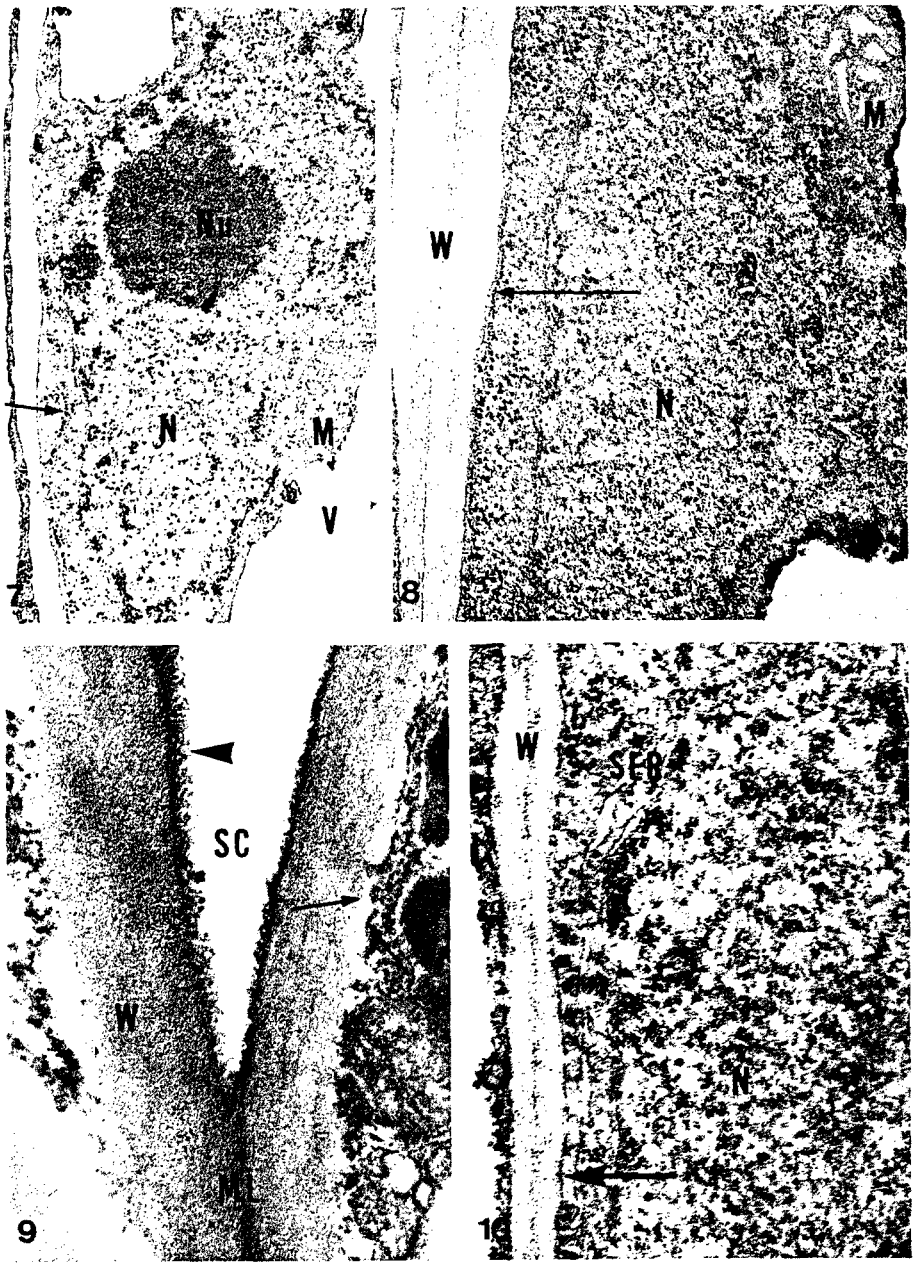
Figs. 7–10. TEM of control, ethylene-treated, and STS-ethylene-treated tissue.

Fig. 7. Abscission zone cell from control *Coleus* explant. Note the elongate nucleus (N) and the nucleolus (Nu), mitochondrion (M), and short segment of rough endoplasmic reticulum (arrow). V = vacuole. ×25,500. **Fig. 8.** *Coleus* abscission zone cell 1 h after beginning of ethylene treatment. Several segments of RER (arrow) and energized mitochondria (M) are visible in the cytoplasm. Note that cell wall (W) is still intact. N = nucleus. ×42,200. **Fig. 9.** Cell of the abscission zone 2 h after ethylene treatment. Note separation of cells along area of the middle lamella (ML) and formation of a separation cavity (SC) between adjacent cells. Note also areas of separation of the plasma membrane and cell wall (W) (arrow) and vesicular or fibrillar material at the separated wall margin (arrowhead). ×31,000 **Fig. 10.** STS-treated tissue after 6 h. Note little evidence of cytological response to ethylene: the cell wall (W) is intact. Note also that there is no obvious disruption of the plasma membrane (arrow) or of the middle lamella, and that profiles of smooth endoplasmic reticulum (SER) are present in the cytoplasm. N = nucleus. ×57,000.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control (air-treated, 0 h) samples (Fig. 10). Some strands of smooth endoplasmic reticulum (SER) were evident in STS-ethylene-treated tissue after 2–6 h of treatment (Fig. 10). There was no apparent increase in RER. Some increase in electron opacity of the tissue was apparent, especially after 4 and 6 h, and occasionally, flocculent material was visible in the vacuole. There was no disruption of membrane systems, and the plasma membrane remained closely appressed to the cell wall (Fig. 10).

Discussion

Physiological and microscopic data indicate that STS pretreatment inhibits ethylene-induced petiole abscission and the corresponding cytological effects of ethylene treatment in *Coleus*. Application of Ag^+ prevents floral, foliar, or fruit abscission in a number of plants (Beyer 1976, Greene 1980, Miranda and Carlson 1981). Other cytological studies have shown that STS or $AgNO_3$ pretreatment can prevent ethylene-induced gum duct formation in *Prunus* fruit (Morrison 1983), proliferation of RER and cytoplasmic breakdown in tobacco flower pedicels (Valdovinos et al. 1981), and parenchyma cell breakdown in "easy-to-shatter" geraniums (Miranda and Carlson 1981).

At the ultrastructural level, ethylene-induced abscission of *Coleus* explants is characterized by an increase in RER and plasma membrane invaginations, dissolution of the middle lamella, some separation of the plasma membrane from the cell wall, and, at later stages, cytoplasmic disorganization. These features are similar to those described in natural abscission of *Coleus* (Bornman 1967, Morre 1968, Halliday and Wangerman 1972a,b, Baird et al. 1978). However, in *Coleus* explants such characteristics are not observed in STS-pretreated tissue.

In our study, delayed treatment with STS progressively reduced its effectiveness in preventing ethylene-induced abscission (Fig. 3). After 4 h of ethylene treatment, STS effectiveness in preventing abscission was considerably reduced. This suggests that events leading to abscission are established between 1 and 4 h after the initiation of ethylene treatment.

Of particular interest is that STS pretreatment appears to prevent the increase in RER observed at 1 h after the initiation of ethylene treatment. Thirtyfold increases in RER have been reported following ethylene treatment in tobacco (Valdovinos et al. 1971), and histochemical studies indicate that peroxidase activity is localized in the proliferated RER in ethylene-treated tobacco (Henry 1975) and bean (Hall and Sexton 1974) abscission zones. Whether the changes in RER in ethylene-treated *Coleus* are related to peroxidase synthesis or not, any reorganization of the endomembrane system could be indicative of a modification of the pattern of cell growth (Sargent and Osborne 1975) and/or related to the metabolite mobilization and protein synthesis/secretion, which are integral to the abscission process (Scott and Leopold 1966, Sexton et al. 1977, Sexton and Roberts 1982). Our study indicates that STS pretreatment prevents the ethylene-induced reorganization of the endomembrane system and the subsequent middle lamellar dissolution.

Further studies will focus on the cytological effects of delayed STS application and the site of STS/ethylene activity within the cell.

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