Plant Physiol. (1993) 101: 49–55

# Rapid Induction of Ion Pulses in Tomato, Cucumber, and Maize Plants following a Foliar Application of L(+)-Adenosine[1]

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**Application of picomole quantities of (+)-adenosine, a plant growth-regulating second messenger elicited by triacontanol, to tomato (*Lycopersicon esculentum* Mill.), maize (*Zea mays* L.), and cucumber (*Cucumis sativa* L.) foliage, increased $Ca^{2+}$, $Mg^{2+}$, and $K^+$ concentrations in the exudate from the stumps of excised plants by 20 to 60% within 5 s after treatment. The change in ionic concentration of the exudate was transitory. When L(+)-adenosine and triacontanol were applied to different tomato plants at the same time, the L(+)-adenosine caused an increase in $Ca^{2+}$ flux within 3 s, whereas a significant increase from triacontanol was not detectable until 5 min after application. This was expected because triacontanol elicits the formation of L(+)-adenosine. The enantiomer of L(+)-adenosine, D(−)-adenosine, had no effect on the cation concentration in tomato and inhibited the effect of L(+)-adenosine at equimolar or lower concentrations. These observations suggest that L(+)-adenosine acts by eliciting a rapidly propagated signal that increases the concentration of several ions in the apoplast. We postulate that modulations in apoplastic ion concentration, especially increases in $Ca^{2+}$ concentration, constitute a mechanism by which plants regulate metabolic activity and growth in response to certain stimuli.**

Since the discovery of the plant growth-regulating properties of TRIA, a primary alcohol that is a natural constituent of plant waxes, and its second messenger L(+)-adenosine, the rapid response kinetics to these compounds have been enigmatic (Ries and Wert, 1988; Ries, 1991).

TRIA increased the dry weight, free amino acids, reducing sugars, and soluble protein of rice (*Oryza sativa* L.) and maize (*Zea mays* L.) plants within 5 min (Ries, 1991). TRIA also elicited the appearance of L(+)-adenosine in the roots of plants whose shoots were sprayed with nanomolar concentrations within 1 min (Ries and Wert, 1988). This was the first evidence that L(+)-adenosine occurred in nature. Synthetic L(+)-adenosine increased the rate of growth of rice seedlings, as measured by total dry weight gain, by more than 50% within 24 h of foliar application of 0.01 to 100.0 $\mu g\ L^{-1}$ ($3.7 \times 10^{-11}$ to $10^{-7}$ M), whereas D(−)-adenosine did not affect plant growth (Ries, 1991).

Several different types of tests have indicated that the TRIA/L(+)-adenosine action may be physical in nature. For example, octacosanol applied to the shoots or roots of rice seedlings inhibited the activity of TRIA applied to the opposite plant part, providing it was applied 1 min prior to TRIA application (Ries and Wert, 1988). TRIA applied to oat (*Avena sativa* L.) or tomato (*Lycopersicon esculentum* Mill.) shoots connected to rice roots by a 4-mm water column also resulted in the appearance of L(+)-adenosine (TRIM) in rice roots (Ries and Wert, 1988).

In an attempt to determine other physiological responses to TRIA in addition to the elicitation of L(+)-adenosine, 20- to 25-d-old tomato seedlings were sprayed with water or TRIA, and after 1 min the plants were excised. Analysis of the diffusate from the excised shoots, as determined by HPLC and atomic absorption spectrophotometry, indicated large concentration differences in organic compounds and inorganic cations (unpublished data). The largest differences were in the cation concentration of the exudate from the stump of the excised tomato plant. Thus, the objective of this research was to use this observation to further elucidate the mode of action for the rapid responses of plants to TRIA and L(+)-adenosine.

We present here evidence that foliar applications of both of these compounds at nanomolar concentrations cause rapid changes in soluble $Ca^{2+}$, $Mg^{2+}$, and $K^+$ concentrations within xylem exudates from the stumps of excised stems and leaves.

## MATERIALS AND METHODS

### Plant Growth and Treatment

Tomatoes (*Lycopersicon esculentum* Mill. cv Sunny), cucumbers (*Cucumis sativa* L. cv Flurry), and maize (*Zea mays* L. cv Pioneer 3780) were grown in a greenhouse with approximately 16 h of supplemental light (700 $\mu mol\ s^{-1}\ m^{-2}$, metal halide) daily. Seeds were planted in 15-cm diameter clay pots containing a soil mix, and the plants were thinned to two or three per pot 8 to 10 d after emergence. Soluble fertilizer (20 N-8.6 P-16.6 K; 1.0 g $L^{-1}$ Peters 20–20–20, W.R. Grace and Co., Fogelsville, PA) was applied once or twice after planting and again prior to treatment. The pots were labeled, randomized for treatments within blocks, and isolated from each other on the greenhouse bench. They were not disturbed for several hours prior to initiation of the treatments.

Experiments were conducted so that the treatments were unknown to the experimenters until after the tests were

[1] This research was supported by the Michigan Agricultural Experiment Station.

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Abbreviations: D(−)-adenosine, 9-β-D-ribofuranosyl,9-H-purine-6-amine; L(+)-adenosine, 9-β-L-ribofuranosyl,9H-purine-6-amine; TRIA, triacontanol; TRIM, second messenger elicited by TRIA and later identified as L(+)-adenosine.

completed, including ion analysis. A minimum of three replicates of two to three plants per pot were used for each treatment. Prior to treatment, the plants were enclosed with cardboard on three sides and the top to prevent the mist from the sprayer (an adjustable linear polyethylene aerosol "Trigger" sprayer; Scientific Products, Chicago, IL) from contacting neighboring plants. The plants were sprayed lightly with solution to drip. For example, 26-d-old tomato plants retained about 350 μL of solution as measured gravimetrically. All experiments were initiated 8 to 12 h into the light period, the optimum time of treatment for L(+)-adenosine (Ries and Wert, 1992). The plants were sprayed with synthetic TRIA (Procter and Gamble); L(+)- and D(−)-adenosine (>99% pure by HPLC analysis) were obtained from Vigoro (Winter Haven, FL) and Aldrich (Milwaukee, WI), respectively.

### Sampling and Analysis

To collect exudate, the shoots were excised with a razor blade 2 to 3 cm below the cotyledonary node. A minimum of two individuals were involved in the application of the foliar treatments, measurement of time, and excision of the stems. Exudate from plants within a single replicate (block) was, however, collected by only one person. In several tests, 1.5 cm of dry vermiculite was placed on top of the soil so that the excised stumps were level with the vermiculite. This prevented the chemical spray from possibly entering the plant through the soil or stem. The exudates from the stumps of the excised shoots were collected with adjustable micropipettes (Eppendorf, Brinkman, Westbury, NY), usually in successive 10- or 20-μL aliquots after excision. Studies using maize plants where the shoot was excised and joined together with latex tubing containing water were carried out as previously described (Ries and Wert, 1988). This procedure took 2 to 5 min. The plants were excised again about 1 cm below the original excision within 5 s of application of water or L(+)-adenosine.

The exudate from a single plant or the combined exudates from two or three plants within an individual pot were diluted to a final volume of 5.0 mL with deionized water. An aliquot was added to a solution of $LaCl_3$ (1000 mg $L^{-1}$) for $Ca^{2+}$ and $Mg^{2+}$ analysis or CsCl (1000 mg $L^{-1}$) for $K^+$ analysis by atomic absorption/emission spectrophotometry (Video 12, Instrumentation Lab, Wilmington, MA).

## RESULTS

### Cation Pulses Elicited by L(+)-Adenosine

L(+)-Adenosine increased the concentration of $Ca^{2+}$ and $Mg^{2+}$ in the exudate by 0.8 mM or more within 5 s of treatment (Table I). A quadratic trend (statistically significant) dose response was obtained with an optimum concentration of L(+)-adenosine at 100 μg $L^{-1}$. The rate of exudate flow was similar between treatments. For example, 20- to 24-d-old tomato seedlings exuded from 8 to 12 μL $min^{-1}$ $plant^{-1}$ depending on the growing environment.

Several experiments showed that ion concentration varied in stem exudates from tomato seedlings with time elapsed between treatment and excision and with time of exudate collection after excision. When the exudate was collected from plants excised 5 s after spraying with L(+)-adenosine, the first 20 μL contained about 40% more $Ca^{2+}$ and 20% more $Mg^{2+}$ than exudate from controls sprayed with water (Fig. 1). These same exudates from L(+)-adenosine plants contained significantly less $K^+$ compared to control plants. However, $K^+$ concentration increased dramatically and exceeded the control by more than 2 mM in the fourth, fifth, and sixth 20-μL fractions (Fig. 1). In contrast, when stem excision was delayed for 24 min, $K^+$ concentrations were less in exudates from the control than from L(+)-adenosine-treated plants. The $Ca^{2+}$ and $Mg^{2+}$ concentrations in the exudates of both controls and treatments increased in successive fractions, whereas the $K^+$ concentration decreased in the controls. Twenty-four minutes after treatment with L(+)-adenosine, there was little difference in $Ca^{2+}$ and $Mg^{2+}$ concentration in exudates from controls and treatments, which indicated that L(+)-adenosine had caused a transient increase in ion concentration (Fig. 1).

**Table I.** *Concentration of $Ca^{2+}$ and $Mg^{2+}$ in exudate collected from the basal stump of 23-d-old tomato seedlings sprayed with different concentrations of L(+)-adenosine 5 s prior to being severed just below the cotyledonary node*

Each observation is the mean of 20-μL samples from two plants (40 μL) in each of three replicates. The *F* value for the quadratic trends of $Ca^{2+}$ and $Mg^{2+}$ with L(+)-adenosine concentration is significant at $P \leq 0.01$.

| L(+)-Adenosine | Ion Concentration | |
|---|---|---|
| | $Ca^{2+}$ | $Mg^{2+}$ |
| μg $L^{-1}$ | mM | |
| 0.0 | 1.34 | 1.88 |
| 1.00 | 1.63 | 1.92 |
| 10.0 | 1.77 | 2.11 |
| 100 | 2.21 | 2.67 |
| 1000 | 1.65 | 2.07 |
| LSD 0.05 | 0.42 | 0.36 |
| LSD 0.01 | 0.62 | 0.53 |

In a study where tomato shoots were excised 5 s, 1 d, and 7 d after treatment with water or L(+)-adenosine, only the 5-s L(+)-adenosine treatment showed significantly higher $Ca^{2+}$, $Mg^{2+}$, and $K^+$ concentrations (Table II). In contrast, the $K^+$ concentration was higher in the controls than in the L(+)-adenosine plants harvested 1 and 7 d after treatment. Plants from the same population that showed the rapid cation response (5 s) to L(+)-adenosine also grew more rapidly than controls, as measured by shoot dry weight 1 and 7 d after treatment (Table II).

### Effect of TRIA on Ion Pulse

Because TRIA elicits L(+)-adenosine production, it follows that when the compounds are applied separately, the L(+)-adenosine should act more rapidly than TRIA. When TRIA or L(+)-adenosine was applied to the foliage of tomatoes and the plants were excised at different times after application, L(+)-adenosine caused a significant increase in $Ca^{2+}$ concentration in exudate from stumps of excised plants within 3 s

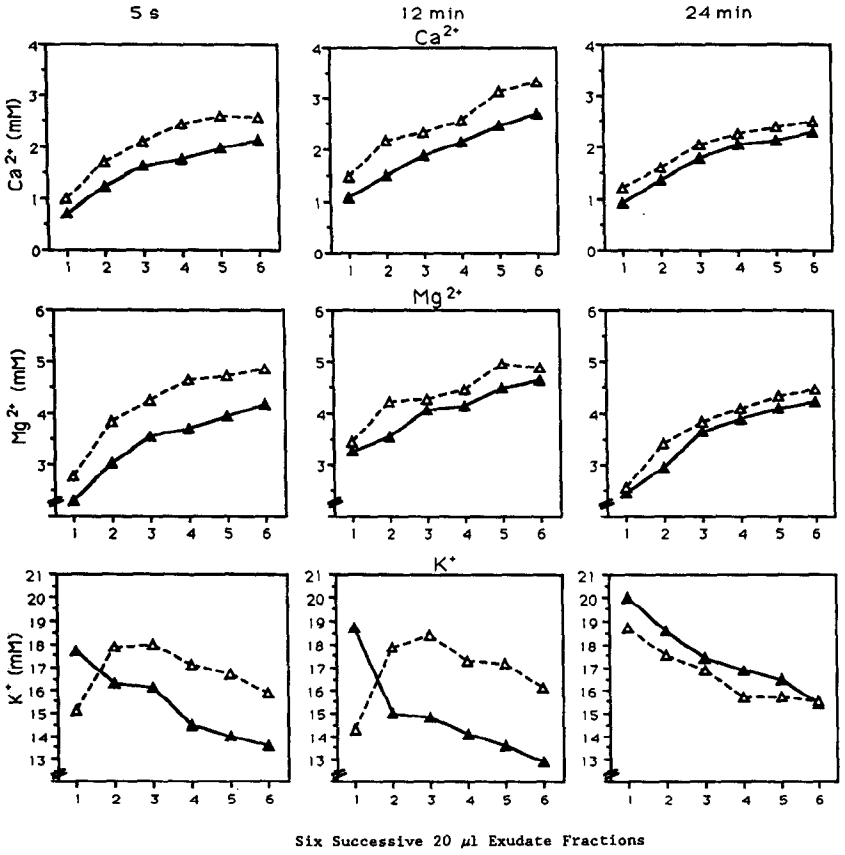



**Figure 1.** Cation concentrations in successive 20-µL exudate fractions from 24-d-old tomato plants sprayed at the same time and excised at 5 s, 12 min, or 24 min after treatment with $H_2O$ (▲) or 100 µg $L^{-1}$ L(+)-adenosine (△). Each observation is the average of two plants in each of three replicates. The *F* values for the main effect of L(+)-adenosine versus control were significant at $P \leq 0.01$ for all $Ca^{2+}$ times and for 5 s of $Mg^{2+}$. The *F* values for the difference in $K^+$ concentration between control and L(+)-adenosine treatments with different exudate fractions were significant at $P \leq 0.01$ for both the 5-s and 12-min treatments.

of application, whereas TRIA required more than 30 s for a similar response (Fig. 2).

### L(+)-Adenosine Activity Inhibited by D(−)-Adenosine

Various controls were used to rule out artifacts in the protocol. Perhaps the best evidence for the lack of artifacts was obtained from application of synthetic D(−)-adenosine, the enantiomer of L(+)-adenosine. Tomatoes were excised 5 s after spraying with 25 different concentration combinations of L(+)-adenosine and D(−)-adenosine. Concentrations of L(+)-adenosine that increased cation pulses were inhibited by as little as 0.01 µg $L^{-1}$ of D(−)-adenosine (Fig. 3). All concentrations of L(+)-adenosine (up to 1000 µg $L^{-1}$) were inhibited by 10.0 µg $L^{-1}$ of D(−)-adenosine. Thus, D(−)-adenosine, the primary form of adenosine found in plants, inhibits the activity of L(+)-adenosine when the two are applied together exogenously. Further research will be necessary to determine the specific mode of inhibition.

### Other Treatments to Show the Rapid Effect of L(+)-Adenosine on Ion Pulses

Maize seedlings were excised and joined together by a 4.0-mm column of water 10 mm above the maize crown within 2 min of spraying with either water or L(+)-adenosine. The

**Table II.** *Relationship of growth of tomatoes (24-d-old) with cation content of exudate from stumps of excised shoots at different times after treatment with L(+)-adenosine*

Each value is the mean of six replicates with four plants per replicate for both dry weight and exudates. Cation concentrations are based on four 40-µL samples from each of four plants (160 µL).

| Time after Treatment | L(+)-Adenosine | Dry Weight | Cation Concentration | | |
|---|---|---|---|---|---|
| | | | $Ca^{2+}$ | $Mg^{2+}$ | $K^+$ |
| | *100 µg $L^{-1}$* | *mg/shoot* | | *mM* | |
| 5 s | 0 | 154 | 1.91 | 5.43 | 11.5 |
| 5 s | + | 155 | 2.22[b] | 7.13[b] | 14.2[b] |
| 1 d | 0 | 170 | 2.28 | 5.92 | 17.9 |
| 1 d | + | 185[a] | 2.28 | 5.62 | 15.2[b] |
| 7 d | 0 | 499 | 2.02 | 3.08 | 17.4 |
| 7 d | + | 537[b] | 2.03 | 3.08 | 15.7[a] |

[a, b] *F* value for comparison with control significant at $P \leq 0.05$ and 0.01, respectively.

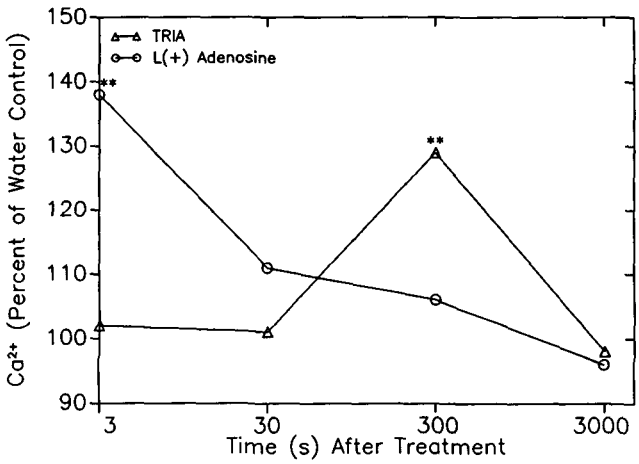



**Figure 2.** The $Ca^{2+}$ concentration in the exudate (40 $\mu$L from each of three plants) from stems of 29-d-old tomato seedlings excised at different times after application of TRIA (1.0 $\mu g\ L^{-1}$) or L(+)-adenosine (100 $\mu g\ L^{-1}$). **$F$ value for comparison of treatment with water-sprayed control significant at $P \leq 0.01$.

exudate from those plants sprayed with L(+)-adenosine contained 43% more $Ca^{2+}$, 32% more $Mg^{2+}$, and 31% more $K^+$ than exudate from water-sprayed controls (Table III). This study indicated that the signal that elicited the increased pulse of cations was able to rapidly transverse a 4.0-mm gap of water joining the shoots of the excised maize seedlings, thus obviating the possibility of diffusion or translocation.

Single leaves centrally positioned on approximately 0.5-m long cucumber plants were sprayed with either water or 100 $\mu g\ L^{-1}$ of L(+)-adenosine. Subsequently, the stem of the main axis was excised at both basal and apical sites as indicated in Figure 4. Analysis of exudates from the four locations (A-D) showed that the major effect was on the exudate from the base of the plant (A in Fig. 4); however, higher $Ca^{2+}$ concentrations also were found in the exudate from both basipetal and acropetal sides of the apical cut on the main stem (Fig. 4, C and D).

A diffusion experiment using tomato plants was conducted to investigate whether the signal elicited by L(+)-adenosine moved both acropetally and basipetally within the plant. Two central leaves, as shown in Figure 5, were sprayed with L(+)-adenosine. An apical and basal leaf were excised within 5 s and placed in 5 mL of water for 5 min. The L(+)-adenosine increased the $Ca^{2+}$ and $Mg^{2+}$ concentrations in the diffusate from the petiole of apical leaves by 40 and 56%, respectively, but decreased the $K^+$ concentration by 21% (Fig. 5). The concentration of all three ions in the diffusate from basal leaves was lower in the L(+)-adenosine treatment.

## DISCUSSION

Exogenously applied L(+)-adenosine is known to elicit numerous physiological responses in plants, including increases in malate dehydrogenase activity (Savithiry et al., 1992) and plant growth (Ries and Wert, 1992). In this study, cation concentrations within exudate solution from detopped plants were modified in a transient manner following foliar applications of L(+)-adenosine (Figs. 1 and 2). Important characteristics of this response are the rapid kinetics occurring within 3 to 5 s of treatment and the magnitude of the changes in exudate $Ca^{2+}$, $Mg^{2+}$, and $K^+$ concentrations up to 40% higher than the controls.

The exudate collected in these experiments is considered to be highly enriched with xylem sap (Ballard, 1960). The concentrations of $Ca^{2+}$, $Mg^{2+}$, and $K^+$ reported here, 1 to 3, 2 to 7, and 12 to 26 mM, respectively, are typical of concentrations found within the xylem (Richardson et al., 1982; Pate, 1989). In contrast, cytosolic $Ca^{2+}$ concentrations have been determined to be much lower, 200 to 400 nM (Evans et al., 1991), whereas that of cytosolic $K^+$ is several fold higher, >100 mM (Lauchli and Pflüeger, 1978), than that in the xylem exudate. This suggests that the extent of contamination by intracellular solutes from mechanically damaged cells upon excision was minimal. It is also highly unlikely that phloem sap comprised a significant percentage of the exudate. $Ca^{2+}$ and $Mg^{2+}$ are present in phloem sap at relatively low concentrations (Richardson et al., 1982). Thus, we believe that the cation concentration changes, measured in response to L(+)-adenosine treatment, occurred within the stem apoplast.

Changes in xylem solution ionic concentration have been attributed to fluctuations in volume flow rate and/or in ion flux rate from or into the symplast (Vaadia, 1960; Armstrong and Kirkby, 1979; Hocking, 1980). No significant differences in volume exudation rate were found between L(+)-adenosine treatments and controls in this study. In addition, the kinetics of the response were so rapid that it is doubtful that changes in the activity of plasma membrane transporters could account for the increases in extracellular $Ca^{2+}$ and $Mg^{2+}$ and the decrease in $K^+$. Less than 2 min were necessary to collect the first 20-$\mu$L fraction of exudate following excision in which

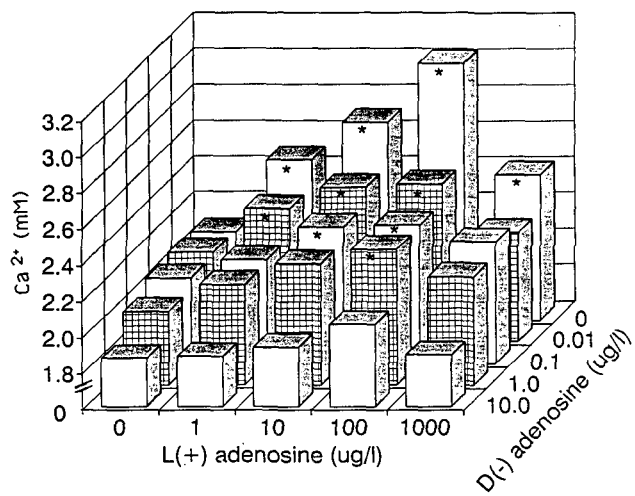



**Figure 3.** Inhibition by D(−)-adenosine of the L(+)-adenosine enhancement of $Ca^{2+}$ concentration in exudate solution collected from the basal stump of excised 28-d-old tomato seedlings. Each point is an average of 40 $\mu$L from two replicates of three plants each. The $Mg^{2+}$ and $K^+$ results were similar to $Ca^{2+}$. Asterisks (*) indicate that means were significantly different from control at $P \leq 0.05$.

**Table III.** *Concentration of cations in exudates of 25-d-old maize seedlings cut 10 mm above crown and joined together by a 4-mm column of water prior to foliar treatment with L(+)-adenosine (100 μg $L^{-1}$) or water*

Plants were cut below the tubing within 5 s of spraying. Each observation is a mean of 40-μL samples from three plants (120 μL) in each of five replicates.

| Treatment | Cation Concentration | | |
|---|---|---|---|
| | $Ca^{2+}$ | $Mg^{2a}$ | $K^a$ |
| | mM | | |
| Water spray or whole plants | 0.54 | 1.29 | 17.9 |
| L(+)-Adenosine on whole plants | 0.78[b] | 1.84[a] | 19.5[a] |
| Water on cut plants with 4-mm gap | 0.59 | 1.45 | 20.2 |
| L(+)-Adenosine on cut plants with 4-mm gap | 0.85[b] | 1.91[a] | 26.4[a] |

[a, b] *F* value for comparison with water controls significant at $P \leq 0.05$ and 0.01, respectively.

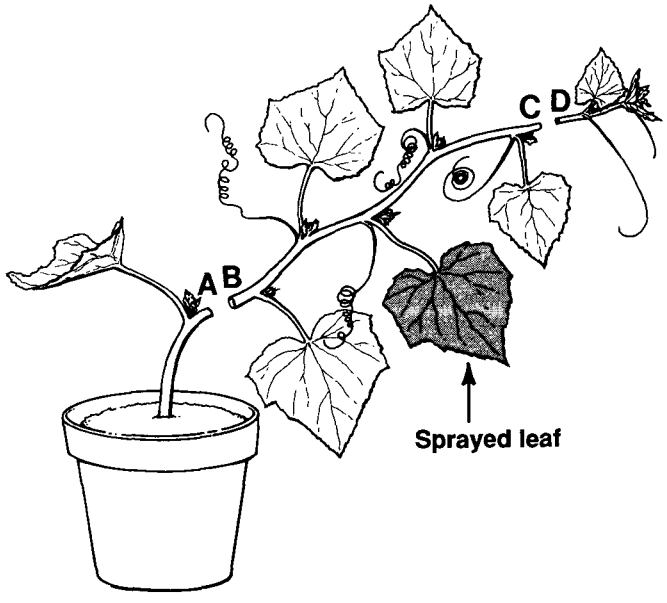
differences in ionic concentration were measured. Also, the chemical potential gradients between the apoplast and the cytosol of stem tissue cells would not be conducive to rapid passive effluxes of $Ca^{2+}$ and $Mg^{2+}$ and an influx of $K^+$, as was suggested by the time course trends in concentration.

The pool of $Ca^{2+}$ and $Mg^{2+}$ contributing to the concentration increase within the xylem solution upon foliar treatment with L(+)-adenosine could not be determined from this study. One potential pool of cations might be the extracellular Donnan phase within the cell wall and the external surface of the plasma membrane (Demarty et al., 1984). The cell wall in stem tissue has a relatively high cation exchange capacity due to the large amount of xylem tissue. A release of $Ca^{2+}$ and $Mg^{2+}$ into the solution phase might be the result of acidification of the apoplast. Alternatively, an intracellular pool of $Ca^{2+}$, which could supply $Ca^{2+}$ indirectly to the apoplast, might be the ER (Buckhout, 1984). Transport of the $Ca^{2+}$ from the ER to the apoplast would necessitate movement across two membranes and, thus, would be expected to be relatively slow.

The mechanism by which L(+)-adenosine elicits a recompartmentation of cations within stem tissue is not evident. It is also quite puzzling as to why an exogenous application of D(−)-adenosine has an inhibitory effect on this response when D(−)-adenosine is the predominant endogenous form of adenosine in plants. In rice roots, for example, approximately 99% of the 125 μg of adenosine $g^{-1}$ dry weight present within the tissue is in the form of D(−)-adenosine (Ries, 1991). A similar situation exists with TRIA, which is inhibited by octacosanol at concentrations of $10^{-12}$ M or less when they are applied together exogenously (Jones et al., 1979). Octacosanol is the predominant long chain alcohol in plants. The most obvious explanation for the lack of an apparent inhibitory effect of endogenous D(−)-adenosine would be that it is localized within a subcellular compartment that is distinct from the site of action of the exogenously applied L(+)-adenosine.

In summary, this study demonstrates that foliar applications of picomole quantities of L(+)-adenosine to apical leaves elicit within seconds a transitory change in $Ca^{2+}$, $Mg^{2+}$, and $K^+$ concentration within the solution phase of the stem apoplast. The fact that the affected tissue is distant (>10 cm) from the treated leaves suggests the involvement of a biophysical signaling system within the plant. It is not clear if the observed changes in ionic concentration are an integral part of the signaling system or a secondary response.

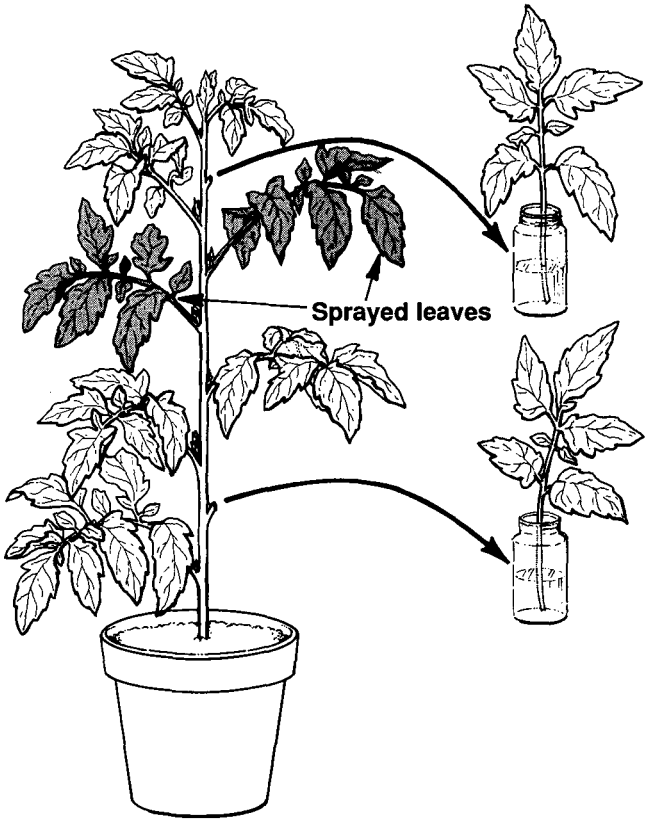
The rapid transient change in extracellular ionic concentration of the magnitude observed in this study is of physiological importance. Modulations in the ionic environment of either leaf or root cells in response to a stimulus would alter the electrochemical potentials across the plasma membrane of those cells. An increased influx of $Ca^{2+}$ into the cytoplasm in response to transient increases in apoplastic $Ca^{2+}$ concen-




| Ion | Treatment | Position | | | |
|---|---|---|---|---|---|
| | | A | B | C | D |
| | | mM | | | |
| $Ca^{2+}$ | $H_2O$ | 2.59 | 1.13 | 0.70 | 0.65 |
| | L(+) | 5.21[b] | 0.84 | 1.13[a] | 1.11[a] |
| $Mg^{2+}$ | $H_2O$ | 3.96 | 3.56 | 2.75 | 2.30 |
| | L(+) | 5.50[b] | 3.15 | 3.28[b] | 2.92[b] |
| $K^+$ | $H_2O$ | 50.0 | 88.1 | 80.7 | 59.4 |
| | L(+) | 64.5[b] | 80.9 | 79.5 | 72.8 |

[a, b] *F* value for comparison of L(+)-adenosine with $H_2O$ control significant at $P \leq 0.05$ and 0.01, respectively.

**Figure 4.** Exudate (10 μL from each of two plants) from the excised stems of 31-d-old cucumber seedlings after a single central leaf was sprayed with $H_2O$ or 100 μg $L^{-1}$ of L(+)-adenosine; plants were excised at basal and apical ends within 5 s. The *F* value for interaction of position on the stem and control versus L(+)-adenosine is significant at $P \leq 0.01$ and $\leq 0.05$ for $Ca^{2+}$ and $K^+$, respectively. Each observation is the mean of six single plant replicates. L(+), L(+)-Adenosine.




| Treatment | Eluent | | |
|---|---|---|---|
| | $Ca^{2+}$ | $Mg^{2+}$ | $K^+$ |
| | *nmol $g^{-1}$ dry weight of leaflets* | | |
| Apical | | | |
| $H_2O$ control | 35[a] | 68[a] | 611[b] |
| L(+)-adenosine | 49 | 106 | 480 |
| Basal | | | |
| $H_2O$ control | 79 | 108 | 505 |
| L(+)-adenosine | 69 | 62 | 380 |

[a] *F* value for interaction of position × treatment significant at $P \leq 0.01$. [b] *F* value for comparison of main effect of $H_2O$ control versus L(+)-adenosine significant at $P \leq 0.01$.

**Figure 5.** The quantity of ions in the eluent from excised leaves of 50-d-old tomato plants whose central two leaves were sprayed with 100 $\mu g\ L^{-1}$ of L(+)-adenosine less than 5 s before the leaves were excised. The petioles of the excised leaves were placed in 5.0 mL of deionized water for 5 min. Each observation is the mean of 10 single-plant replicates.

tration, as reported here, would modulate the activity of important regulatory enzymes within cells by binding to such receptor proteins as calmodulin (Marmé, 1986; Evans et al., 1991).

Relative to the mode of action of exogenously applied TRIA and L(+)-adenosine, it is postulated that both compounds move rapidly through the leaf cuticle to the plasma membrane of epidermal cells. TRIA then elicits the formation of L(+)-adenosine. This study suggests that L(+)-adenosine triggers a rapidly transmitted signal within whole plants that results in a transient increase in apoplastic ion concentration within stem tissue. These results are consistent with and offer supportive evidence for the anomalous rapid responses of plants to both TRIA and L(+)-adenosine (Ries, 1991).

Received June 30, 1992; accepted September 8, 1992.


## LITERATURE CITED

**Armstrong MJ, Kirby EA** (1979) Estimation of potassium recirculation in tomato plants by comparison of the rates of potassium and calcium accumulation in the tops with their fluxes in the xylem stream. Plant Physiol **63:** 1143–1148

**Ballard EG** (1960) Transport in the xylem. Annu Rev Plant Physiol **11:** 141–166

**Buckhout TJ** (1984) Characterization of $Ca^{2+}$ transport in purified endoplasmic reticulum membrane vesicles from *Lepidium sativum* L. roots. Plant Physiol **76:** 962–967

**Demarty M, Morvan C, Thellier M** (1984) Calcium and the cell wall. Plant Cell Environ **7:** 441–448

**Evans DE, Briars SA, Williams LE** (1991) Active calcium transport by plant cell membranes. J Exp Bot **42:** 285–303

**Hocking PJ** (1980) The composition of phloem exudate and xylem sap from tree tobacco (*Nicotiana glauca* Grah.). Ann Bot **45:** 633–643

**Jones J, Wert VF, Ries SK** (1979) Specificity of 1-triacontanol as a plant growth stimulator and inhibition of its effects by other long-chain compounds. Planta **144:** 277–282

**Läuchli A, Pflüeger R** (1978) Potassium transport through plant cell membranes and metabolic role of potassium in plants. *In* Potassium Research-Reviews and Trends. Proceedings of the 11th Congress of the International Potash Institute, Bern, Switzerland, pp 111–163

**Marmé D** (1986) The role of calcium in the regulation of plant metabolism. *In* AJ Trewavas, eds, Molecular and Cellular Aspects of Calcium in Plant Development. Plenum Press, New York, pp 1–8

**Pate JS** (1989) Origin, destination and fate of phloem solutes in relation to organ and whole plant functioning. *In* DA Baker, JA

Milburn, eds, Transport of Photoassimilates. Longman Scientific & Technical, England, pp 138–164

**Richardson PT, Baker DA, Ho LC** (1982) The chemical composition of cucurbit vascular exudates. J Exp Bot **33:** 1239–1247

**Ries SK** (1991) Triacontanol and its second messenger 9-β-L(+)-adenosine as plant growth substance. Plant Physiol **95:** 986–989

**Ries SK, Wert VF** (1988) Rapid elicitation of second messengers by nanomolar doses of triacontanol and octacosanol. Planta **173:** 79–87

**Ries SK, Wert VF** (1992) Response of maize and rice to 9-β-L(+) adenosine applied under different environmental conditions. Plant Growth Regul **11:** 69–74

**Savithiry S, Wert V, Ries S** (1992) Influence of 9-β-L(+) adenosine on malate dehydrogenase activity in rice. Physiol Plant **84:** 460–466

**Vaadia Y** (1960) Autonomic diurnal fluctuations in rate of exudation and root pressure of decapitated sunflower plants. Physiol Plant **13:** 701–717