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(54) Title: METHOD FOR THE TREATMENT OF PLANT PARTS TO IMPROVE QUALITY (57) Abstract A method for the treatment of a plant with L(+)adenosine to improve the quality of the parts at the extremities of the plant, such as fruit, vegetables (including root vegetables) and leaves. The method includes applying L(+) adenosine to the plant or part of the plant prior to harvest of the fruit or vegetable. Increases in mineral pulses in the terminal parts of the plant produce the increase in quality.		

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-1-

METHOD FOR THE TREATMENT OF PLANT
PARTS TO IMPROVE QUALITYBACKGROUND OF THE INVENTION(1) Field of the Invention

The present invention relates to a method for the treatment of parts of a plant shortly prior to harvest to improve the quality of the parts, particularly the firmness and storage stability of the part, by increasing the cation concentrations at the extremities of the plant. In particular, the present invention relates to the use of L(+)adenosine to increase the firmness and storage stability of fruits and vegetables.

(2) Prior Art

U.S. Patent No. 4,849,012 to Wilson describes the use of triacontanol applied to the trees and fruits to increase the sugar content in oranges. The use of triacontanol and trim to stimulate plant growth is described in U.S. Patent Nos. 4,150,970 to Ries et al and 4,741,754 to Ries. U.S. Patent No. 4,333,758 to Welebir also shows triacontanol compositions.

U.S. Patent No. 5,009,698 to Ries, Wert and Nair, describes the use of L(+)adenosine to stimulate the growth of a plant. The L(+)adenosine is applied early in the growth of the plant and produces significant yield improvements.

Triacontanol (TRIA) is used on millions of hectares to increase crop yield, particularly in Asia. In controlled environment studies, synthetic L(+) adenosine was found to increase the rate of growth of rice seedlings as measured by total dry weight gain, more than 50% within 24 hours of a foliar application of 0.01 to 100.0 $\mu\text{g}\cdot\text{L}^{-1}$ (3.7×10^{-11} to 10^{-7} M) (Ries, S. K., et al., Plant Growth Reg. 9, 263 (1990)). L(+) adenosine has shown promise in early field trials to be more consistent than TRIA.

OBJECTS

It is therefore an object of the present invention to provide a method which increases the firmness, storage stability and shelf life of fruits and vegetables.

It is further an object to provide a method which is simple and economic for use in the field (farm, ranch or grove) or in the greenhouse.

These and other objects will become increasingly apparent by reference to the following description and the drawings.

IN THE DRAWINGS

Figures 1A to 1I show the ion concentration in successive 20 μ l fractions from 24-day-old tomato plants excised at 5 seconds, 12 minutes and 24 minutes after treatment with H_2O or 100 μ g L^{-1} L(+) adenosine. Each observation is average of 2 plants in each of 3 replicates. The F values for the main effect of L(+) adenosine vs. control were significant at $P \leq .01$ for all Ca^{2+} times, and for 5 seconds Mg^{2+} . The F value for the difference in K^+ concentration between control and L(+) adenosine treatments with different exudate fractions was significant at $P \leq .01$ for both the 5 second and 12 minute treatments.

Figure 2 shows a plant which has been cut as described in Table 2 for collecting exudate (10 μ l from each of 2 plants) from the cut stems of 31-day-old cucumber seedlings after single central leaf was sprayed with H_2O or 100 μ g L^{-1} of L(+) adenosine and then plants excised at basal and apical ends within 5 seconds. The F value for interaction of position on the stem and control versus L(+) adenosine significant at $P \leq .01$ and $\leq .05$ for Ca^{2+} and K^+ , respectively. Each observation is the mean of six single plant replicates.

Figure 3 shows a plant which has been cut as shown to determine the concentration of ions in the eluate from excised leaves of 50-day-old tomato plants

whose central 2 leaves were sprayed with $100 \mu\text{g L}^{-1}$ adenosine less than 5 seconds before the leaves were excised and placed in 5.0 ml of distilled water for 5 minutes. Each observation is mean of 10 single plant replicates.

Figure 4 is a bar graph showing the effect of L(+)adenosine (LAD) on the sugar to acid ratio in tomatoes when applied prior to harvest at concentrations of 10 and $100 \mu\text{g L}^{-1}$ versus a control (water).

GENERAL DESCRIPTION

The present invention relates to a method for improving the quality of a part which is to be removed from a plant which comprises: (a) applying L(+)adenosine to the plant before the part is removed in an amount sufficient to increase ion concentrations at extremities of the plant; and (b) harvesting the part soon after the application to thereby improve the quality of the part.

Further, the present invention relates to a method for inhibiting spoilage which is associated with a lack of firmness of a skin of the plant in a harvested part severed from a plant which comprises: applying L(+)adenosine to the plant in an amount sufficient to increase puncture resistance of a skin of the part and to inhibit the spoilage of the part of the plant to be harvested; and harvesting the plant part soon after the application.

Since the discovery of the plant growth regulating properties of triacontanol (TRIA) and later its second messenger $9\text{-}\beta\text{-L}(+)\text{adenosine}$ [(9H-purin-6-amine, $9\text{-}\beta\text{-L}$ ribofuranosyl, L(+) adenosine)], there has been the enigma of the extremely rapid responses (Ries, S. K., Plant Physiol. 95, 986 (1991)). It is now believed that nanomolar concentrations of L(+) adenosine (MW, 267) act by indirectly causing changes in the concentration of several ions within the apoplastic solution phase (xylem solution) of plant tissue.

-4-

The treatment is between 0.1 and 60 days before harvest, preferably 1 to 30 days. Longer times do not appear to produce significant results.

5 The L(+)adenosine can be synthetically prepared as described in U.S. Patent No. 5,009,698. Usually it is in high purity and free of inhibitory compounds. The amount of L(+)adenosine is provided between about 0.01 to 10,000 micrograms per liter in an aqueous solution. The L(+)adenosine is applied in a
10 spray in most instances although other methods can be used.

The present invention results from the fact that the mineral ion fluxes in the plant increase upon application of L(+)adenosine to the plant thereby
15 increasing the levels of these ions in the fruit and vegetables at the extremities of the plant. The increased level of mineral ions in the fruits or vegetables provides a longer shelf life and firmness.

SPECIFIC DESCRIPTION

20 The following Examples 1 to 7 show the increases in mineral ion pulses as a function of time in various plants.

Example 1

Applications of picomole quantities 9- β -L(+) adenosine, the second messenger elicited by
25 triacontanol, to tomato foliage increase Ca^{2+} , Mg^{2+} and K^+ in the exudate from cut stems of tomato and cucumber seedlings by 20 to 60%. The increase in Ca^{2+} and Mg^{2+} from the L(+) adenosine treatment occurred when the
30 plants were cut within 5 s after application in the first 10-20 μl of exudate collected within 1 to 2 minutes after the plants were excised. The increase in the concentration of K^+ in the exudates, due to the L(+) adenosine treatment, did not occur until the 40 to 60 μl
35 fraction of the exudate was collected. When plants were cut at different times after application of L(+) adenosine, the difference in Ca^{2+} and Mg^{2+} concentrations

due to L(+) ado treatment decreased within 12 minutes of application, whereas the difference in K⁺ concentration increased. The difference in concentration of all ions was minimal in exudates taken 25 minutes after the stem was excised and absent after 24 hours.

5 There was a clear response of tomatoes to the dose of L(+) adenosine with an optimum concentration of 100 µg L⁻¹ (3.7 x 10⁻⁷ M). The enantiomer of L(+) adenosine, 9-β-D(-) adenosine had no effect on the ion fluxes in tomato and inhibited the effect of 9-β-L(+) adenosine on ion fluxes at equimolar concentrations.

10 'Sunny' tomatoes (*Lycopersicon esculentum* Mill.) and 'Flurry' cucumbers (*Cucumis sativus* L.) were grown in a greenhouse under approximately 16 hours of 700 µmol·sec⁻¹·m⁻² of supplemental light (metal halide). Several seeds were planted in 15.0 cm diameter clay pots and thinned to 2 or 3 plants 8 to 10 days after emergence. Soluble fertilizer (200 ml of 1.0 g L⁻¹ of 20-20-20 was applied once or twice after planting and again the day prior to treatment. Twenty to 25 days after planting the plants (3-4 true leaves) were watered with nutrient solution again. The pots were labeled, randomized for treatments within blocks and isolated on the greenhouse bench so they were not touching. They were not disturbed prior to the initiation of the experiments.

25 Plants were treated at previously selected random locations on the greenhouse bench, with a minimum of 3 replicates of 2 to 3 plants per treatment. Prior to treatment, the plants were surrounded on 3 sides and the top with cardboard to prevent the mist from the sprayer (an adjustable linear polyethylene aerosol "Trigger" sprayer from Scientific Products Co., Chicago) from contacting neighboring plants. The plants were not sprayed to drip. For example, 26-day-old tomato plants retained about 350 µl of spray. All experiments discussed here were conducted 8 to 12 hours into the

photoperiod because it had been previously shown that plants respond best to L(+) adenosine at this time (Ries, S. K. and V. F. Wert, Plant Growth Reg 10, In press (1991)). The plants were sprayed with synthetic L(+) adenosine, (>99% pure by HPLC analysis) (The Vigoro Corporation, Winter Haven, FL 33882), synthetic D(-) adenosine (>99% pure by HPLC analysis) (Aldrich Corp., Milwaukee, WI 53233).

To collect exudate, the plants were excised with a razor blade 2.0-3.0 cm below the cotyledonary node at the surface of the dry vermiculite. The exudate from the cut stem were collected with adjustable micro pipettes, usually in successive 10 or 20 μ l aliquots, which were available within 1 to 2 minutes after excision. The exudate from all of the shoots (2 or 3) in each pot was placed in 5.0 ml of deionized water. An aliquot of this was added to a solution of LaCl₃ (1000 mg L⁻¹) for Ca²⁺ and Mg²⁺ analysis or CsCl (1000 mg L⁻¹) for K⁺ analysis by atomic absorption/emission spectrophotometry (Video 12, Instrumentation Lab Inc., Wilmington, MA 01887).

Dose response studies showed that the optimum concentration of L(+) adenosine was about 100 μ g L⁻¹ as shown in Table 1.

-7-

Table 1

L(+) adenosine concentration		Ion concentration. (mM)		
	($\mu\text{g L}^{-1}$)	Ca^{2+}	Mg^{2+}	K^{+}
5	0.0	1.34	1.88	18.8
	1.00	1.63	1.92	19.3
	10.0	1.77	2.11	19.0
	100	2.21	2.67	20.9
10	1000	1.65	1.24	19.7
LSD .05		.42	.36	NS
LSD .01		.62	.52	

15 The F value for the quadratic trends of Ca^{2+} and Mg^{2+} with L(+) adenosine concentration is significant at $P \leq .01$. The Ca^{2+} and Mg^{2+} concentration in the exudates was less at 1000 $\mu\text{g L}^{-1}$ of L(+) adenosine. At the optimum concentration each picomole of L(+) adenosine applied to the foliage causes a difference of more than 100 picomoles of Ca^{2+} and Mg^{2+} in the first 20 μl of exudate. There was no significant difference in K^{+} concentration as shown in Table 1.

Example 2

25 Early studies revealed that it was not possible to cut the plants quickly enough to obtain similar ion concentrations in exudates from control plants sprayed with water and those treated with L(+) adenosine. The only "zero time" control that proved to be the same as the water control was to cut the plants and hold them over the cut stems prior to spraying with L(+) adenosine.

35 Several experiments showed that ion concentration varied depending on the time the plant was excised after treatment. When the exudate was collected from plants cut 5 seconds after spraying with either water or L(+) adenosine the first 20 μl contained about 40% more Ca^{2+} and 20% more Mg^{2+} than controls sprayed with water as shown in Figures 1A to 1H. These same exudates

showed significantly less K^+ in the first 20 μ l of exudate from L(+) adenosine treated plants. However, by the fourth 20 μ l fraction the K^+ concentration in the L(+) adenosine treatment was more than 2 mM higher than the control (Figures 1A to 1I). Ca^{2+} and Mg^{2+} concentration at all times increases with successive fractions whereas the K^+ concentration decreases in the controls with successive fractions. Twenty-four minutes after treatment with L(+) adenosine, there is little difference between the ion concentration in controls and treatments, indicating that L(+) adenosine caused a pulse of ions which could not be detected after 24 minutes (Figures 1C, 1F and 1I). In the first 100 μ l of exudate one picomole of L(+) adenosine increased the quantity of Ca^{2+} and Mg^{2+} by more than 2,000 picomoles and K^+ more than 40,000 picomoles. In a study where plants were excised 5 seconds, 24 hours and 48 hours after treatment with water or L(+) adenosine, only the 5 second L(+) adenosine treatment showed significantly higher ion concentrations (data not shown).

Example 3

Various controls were used to establish whether or not there was an artifact in the protocol. Perhaps the best control was synthetic D(-) adenosine the enantiomer of L(+) adenosine. Equimolar concentrations of D(-) adenosine had no effect on the ion concentration in the exudate from the cut stems. In a test where the plants were severed 5 seconds after spraying with 10 μ g L⁻¹ L(+) adenosine or a mixture of L(+) adenosine and D(-) adenosine the average concentration of Ca^{2+} in the water control. L(+) adenosine and the equimolar mixture was 2.54, 3.89 and 2.69 mM, respectively. The F value for the difference of the L(+) adenosine treatment from the other treatments was significant as $P \leq .01$. In another test, a higher concentration of L(+) adenosine was used (100 μ g L⁻¹) to determine if the inhibition by D(-) adenosine

(10 $\mu\text{g L}^{-1}$) could be overcome. The Ca^{2+} concentrations in the H_2O , L(+) adenosine, D(-) adenosine and mixture of L(+) and D(-) adenosine were 1.72, 2.32, 1.62 and 1.84 mM, respectively. The F value for the comparison of the L(+) treatment with others was significant at $P \leq .01$. Further tests with both cucumbers and tomatoes showed that 1.0 $\mu\text{g L}^{-1}$ of D(-) adenosine inhibited the activity of L(+) adenosine at 10, 100 and 1000 $\mu\text{g L}^{-1}$, as measured by Ca^{2+} and Mg^{2+} concentration in the exudate (data not shown). Thus, the primary form of adenosine (D(-) adenosine) found in plants inhibits the activity of L(+) adenosine when the two are applied together exogenously.

Example 4

A single central leaf of cucumber plants which were about 100 cm long was sprayed with either water or 100 $\mu\text{g L}^{-1}$ of L(+) adenosine. Subsequently, the stem was excised at basal and apical sites on the main axis as indicated in Figure 2. Analysis of exudates from the four locations showed that the major effect was on the exudate from the base of the plant, however, higher Ca^{2+} concentrations were measured from both basipetal and acopetal sides of the apical cut of the central stem (Figure 2). The results are shown in Table 2.

Table 2

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

*,**F value for comparison of L(+) with H_2O control significant at $P \leq .05$ and $.01$, respectively.

-10-

Example 5

Two central leaves of tomato plants, as shown in Figure 3, were sprayed with L(+) adenosine and the apical and basal leaves were excised within 5 seconds and placed in distilled water for 5 minutes. The L(+) adenosine increased the Ca^{2+} and Mg^{2+} concentration in the eluant from the petiole of apical leaves by 37 and 14%, respectively, whereas the K^+ concentration was 15% less in the L(+) adenosine treatment (Fig. 4). The concentration of all ions from the eluant of the petioles from basal leaves was lower in L(+) adenosine treatment. The results are shown in Table 3.

Table 3

Eluant

(n moles g^{-1} dry weight of leaflets)

Treatment	Ca^{2+a}	Mg^{2+a}	K^{+b}
Apical			
H ₂ O control	35	68	611
L(+) adenosine	49	106	480
Basal			
H ₂ O	79	108	505
L(+) adenosine	69	62	380

^aF value for interaction of position.

^x treatment significant at $P \geq .01$.

Example 6

When tomato plants were sprayed with L(+) adenosine, and the shoots excised, the difference in concentration of Ca^{2+} and Mg^{2+} in the exudate of controls and L(+) adenosine treatment in the sap exuding from the cut shoots was significantly different for at least 5.0 minutes (data not shown). In cucumber plants it was not possible to obtain more than about 10 μl of sap from each cut because the exudate became too "gummy" to pick up with the pipette.

-11-

Example 7

The stems of tomato plants were severed at different times after application of L(+) adenosine to determine how long the treatment affected the flux of ions in the stem tissue. The concentration of Ca^{2+} and Mg^{2+} decreased linearly from 3 seconds to 200 minutes (Figure 4). These tests indicated that the L(+) adenosine induced a transitory redistribution of ions within stem tissue for 5 minutes after which the concentration of Ca^{2+} and Mg^{2+} the exudate came to equilibrium.

Example 8

Figure 4 shows the results of spraying green tomatoes with L(+) adenosine (10 and 100 μL^{-1}) and then harvesting the tomatoes seven (7) days later. In Figure 4, LAD is L(+) adenosine. BCD is the carrier alone. Figure 4 shows the increase in the sugar to acid ratio in the tomatoes. The green tomatoes were also stored at 80°F (26.7°C) in high humidity. The results are shown in Table 4.

Table 4

Storage Life of Tomatoes

Tomatoes sprayed and picked "Mature green" and stored at 80°F in high humidity.

L(+) adenosine $\mu\text{g/liter}$	Days until 50% of Fruit rotted. Time (Days)
Control	15
10.0	24
100	29

Example 9

Redhaven peach trees, 4 years old with fruit and having a trained open center were treated with L(+) adenosine thirty (30) days before the beginning of harvest (5 days harvest) before harvesting to determine the increase in firmness. A foliar spray of one-half gallon (1.89 liters) was applied to each tree. The

results are shown in Table 5 wherein the "mean" represents the firmness in grams per square centimeter. Each mean is the average of four (4) trees.

Table 5

5	<u>Chemical</u>	<u>Concentration</u> <u>µg/liter</u>	<u>Firmness</u> <u>(gm/cm²)</u>
	Control		9.54
	Triaccontanol	2	9.13
10	L(+) adenosine	2	9.94
	Triaccontanol	18	10.25
	L(+) adenosine	0.5	10.56
	Triaccontanol	10	10.82
	L(+) adenosine	1.0	14.53

15 Thus 1 ppb of L(+) adenosine (trim) produced a 52% increase over the control. For all of the L(+) adenosine treatments of peaches the mean was 11.67 g/cm² or a 22% increase over the control.

Example 10

20 The following Table 6 shows the effect of L(+) adenosine on Ca²⁺ composition of "Flurry" pickling cucumbers in a Field test. The L(+) adenosine was applied twice at 168 hours and 48 hours before harvest at 100 µg per liter each application.

Table 6

25 Effect of L(+) adenosine on Ca²⁺ composition of "Flurry" pickling cucumbers in a field test.

(g Ca²⁺ g⁻¹ dry wt x 1000)

30	<u>Treatment</u>	<u>Pericarp</u>		<u>Endocarp</u>		<u>Total</u>
		<u>Conc</u>	<u>Total</u>	<u>Conc</u>	<u>Total</u>	<u>fruit</u>
	Water control	19.6	849	8.29	116	966
35	100 µg/L L(+) adenosine	19.7	863	9.09	129	992
	F value for comparison	NS	≤.05	≤.05	≤.05	NS

40

The pericarp is between the epidermis and endocarp. The endocarp is around the seed cavity. The

increase in Ca^{2+} prevents "bloaters".

The calcium concentrations (2-4 mM) observed in the exudates in these studies are considered to be of apoplastic origin because the concentrations are several thousand times higher than the 200-400 nM concentrations reported for cytoplasm in plant cells, (Evans, D. E., et al., J. Exp. Bot. 42, 285 (1991)).

There is much evidence for Ca^{2+} acting as a intracellular sensory system for numerous environmental stimuli such as insect and disease invasion, heat and cold shock, touching, wind and rain (Braam, J., et al., Cell 60, 357 (1990); and Mitchell, C. A., et al., J. Amer. Hort. Sci. 100, 161 (1975)). Gibberellic acid has been shown to be related to increases in intracellular free Ca^{2+} in barley aleurone layers, and cytokinins increased intercellular Ca^{2+} by activating plasma membrane ion channels (Schroeder, J. I., et al., The Plant Cell 3, 555 (1991)). It has also been suggested that calcium ions and calmodulin were involved in transduction of signals from the environment which enabled plants to sense and respond to environmental changes (Evans, D. E., et al., J. Exp. Bot. 42, 285 (1991)). However, there was no evidence in the preceding research for the changes in Mg^{2+} and K^+ concentrations we have measured in tomato exudates.

A hypothesis that would explain the present invention is that nanomolar quantities of L(+) adenosine elicit membrane potentials or some other biophysical change, which results in specific release of ions in mmolar quantities from cell walls or other insoluble chelators within the stem and root apoplast. These ions may act in concert to initiate a cascade of enzyme or hormone activity, which results in the observed metabolic, dry weight changes and skin firmness.

It is intended that the foregoing description be only illustrative of the present invention and that

the present invention be limited only by the hereinafter
appended claims.

-15-

I CLAIM:

-1-

A method for improving the quality of a part which is to be removed from a plant which comprises:

5 (a) applying L(+) adenosine to the plant before the part is removed in an amount sufficient to increase ion concentrations at extremities of the plant; and

(b) harvesting the part soon after the application to thereby improve the quality of the part.

-2-

The method of Claim 1 wherein the part is harvested from the plant within 0.1 to 60 days of application of the L(+)adenosine.

-3-

The method of Claim 1 wherein the L(+) adenosine is in water containing between about 0.01 and 10,000 $\mu\text{g/liter}$ of L(+)adenosine.

-4-

The method of Claim 1 wherein the plant part is a fruit or vegetable.

-5-

The method of Claim 4 wherein the plant part is a tomato as the fruit.

-16-

-6-

A method for inhibiting spoilage which is associated with a lack of firmness of a skin of the plant in a harvested part severed from a plant which comprises:

5 (a) applying L(+)adenosine to the plant in an amount sufficient to increase puncture resistance of a skin of the part and to inhibit the spoilage of the part of the plant to be harvested; and

10 (b) harvesting the plant part soon after the application.

-7-

The method of Claim 6 wherein the L(+) adenosine is applied in water.

-8-

The method of Claim 7 wherein the L(+) adenosine in water containing between about 0.01 and 10,000 $\mu\text{g}/\text{liter}$ of L(+)adenosine.

-9-

The method of Claim 6 wherein the part is a fruit or vegetable.

-10-

The method of Claim 9 wherein the part is a tomato.

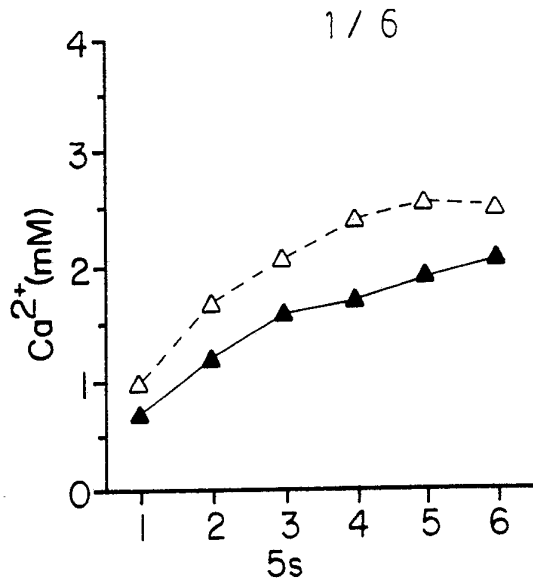


FIG. 1A

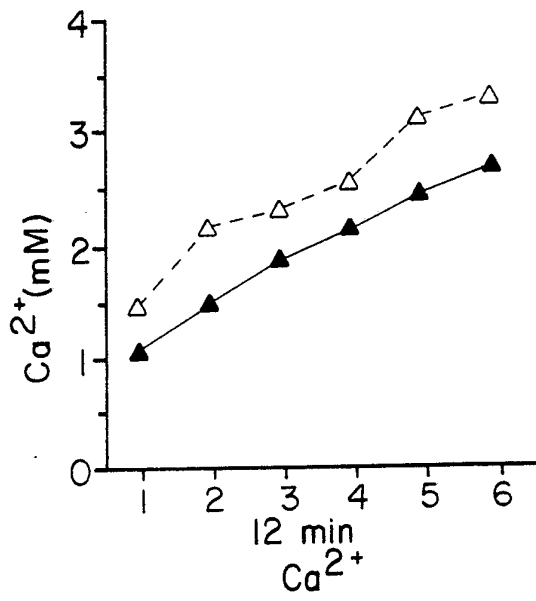


FIG. 1B

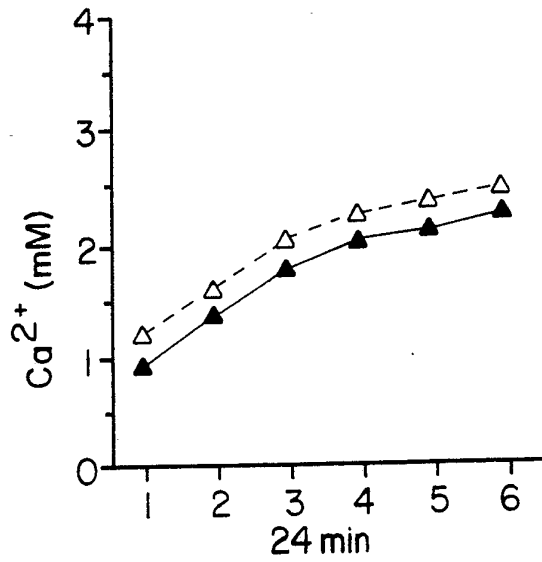


FIG. 1C

2 / 6

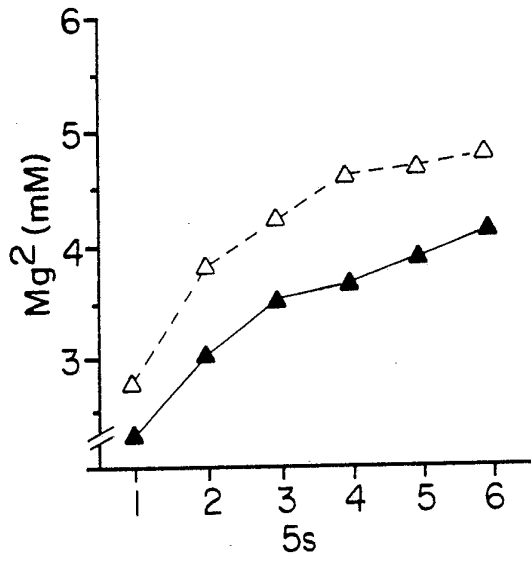


FIG. 1 D

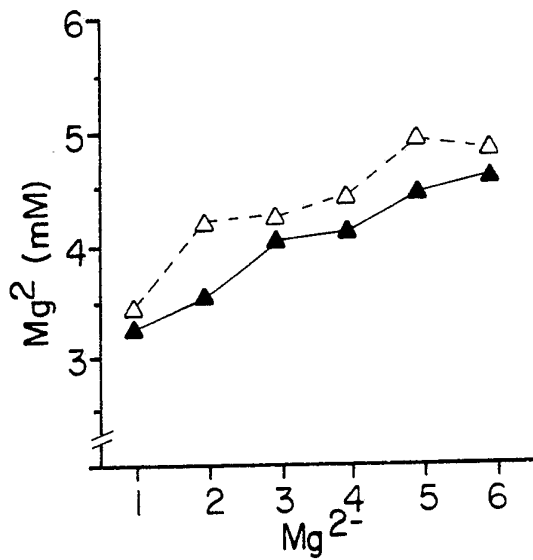


FIG. 1 E

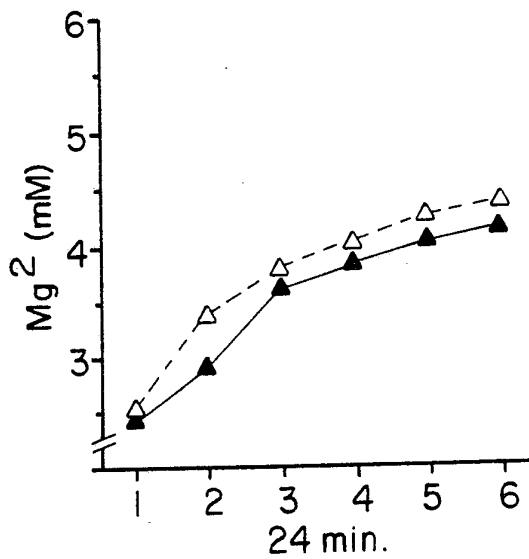
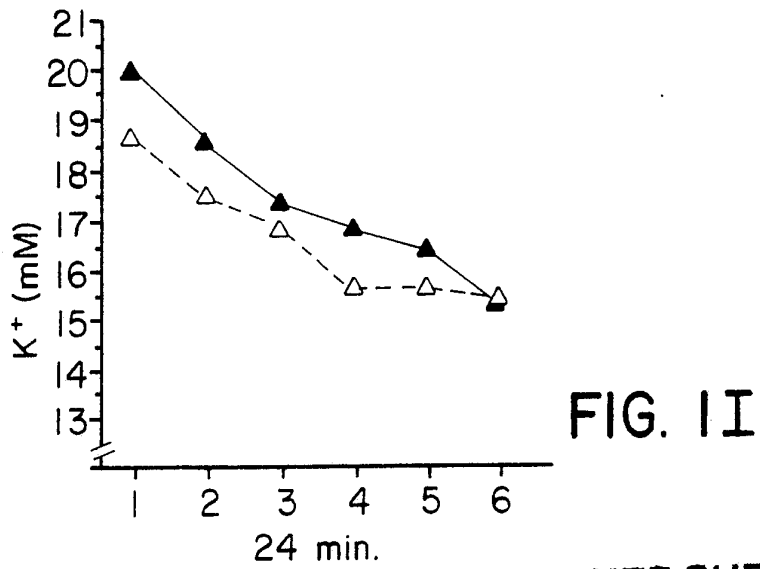
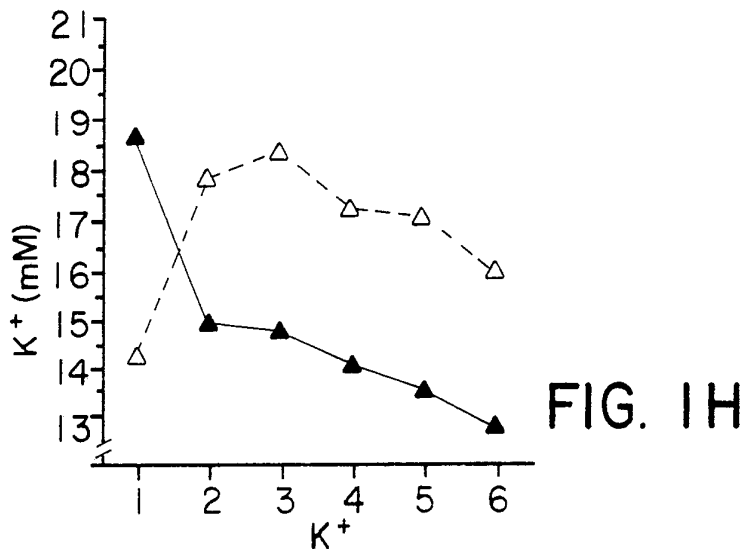
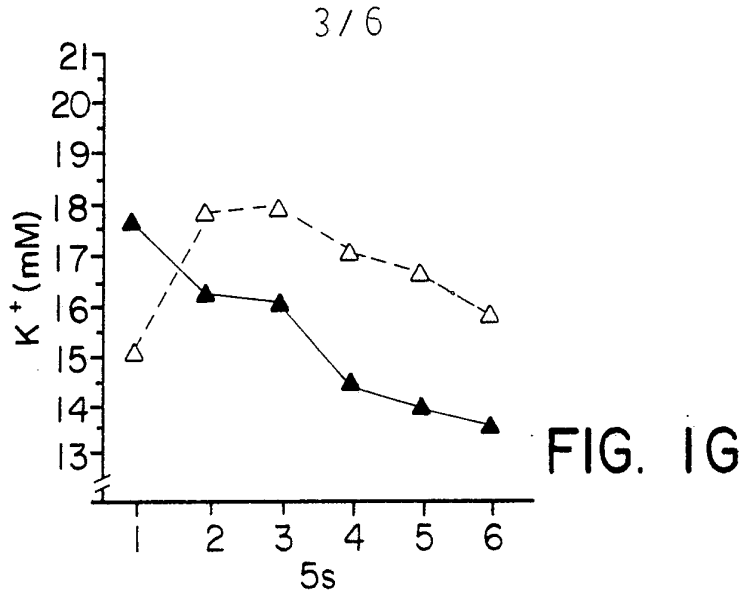


FIG. 1 F



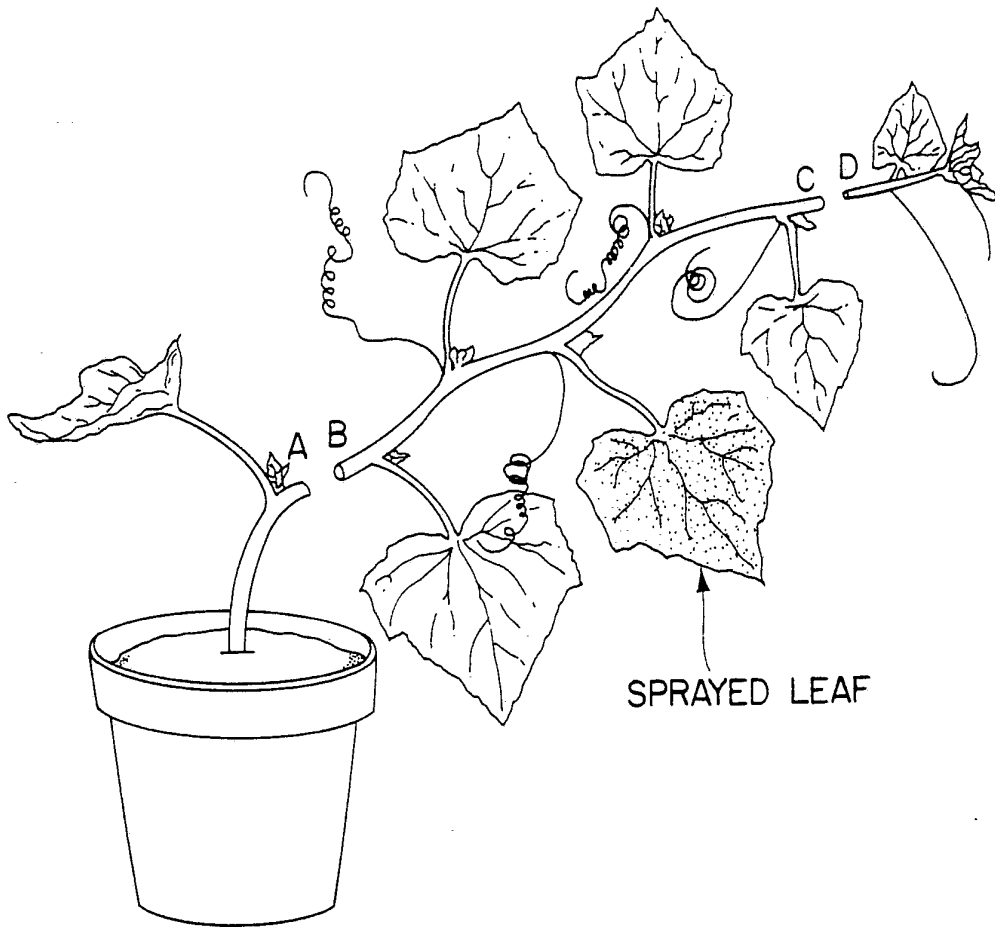


FIG. 2

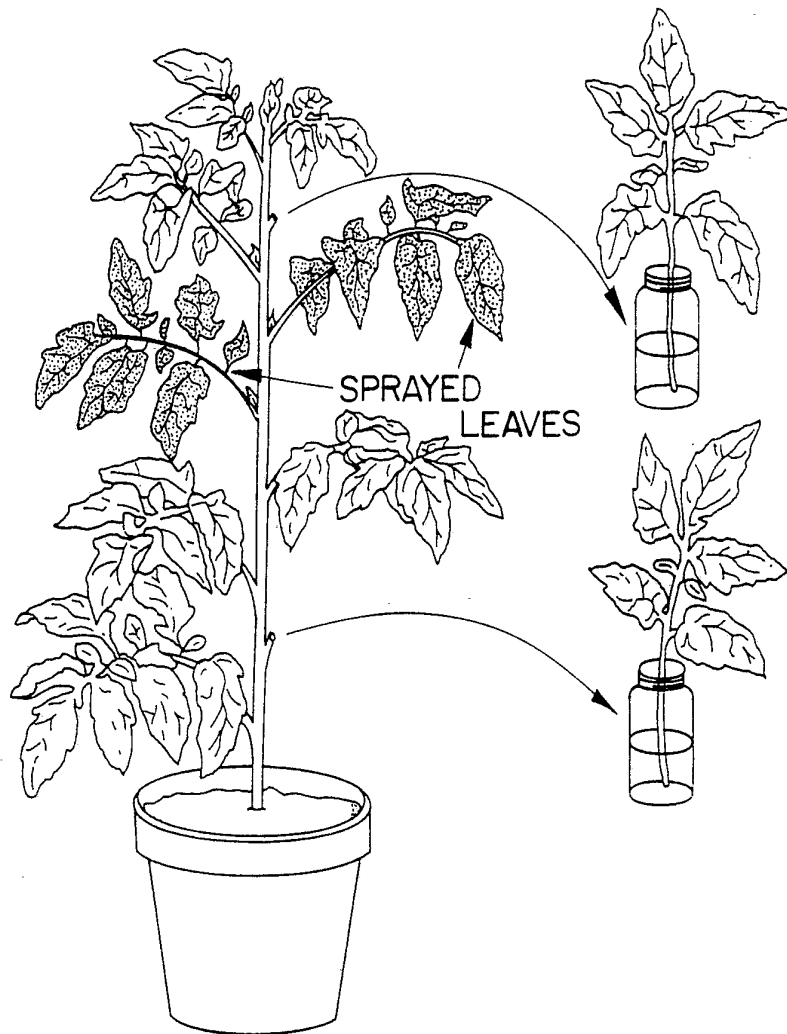


FIG. 3

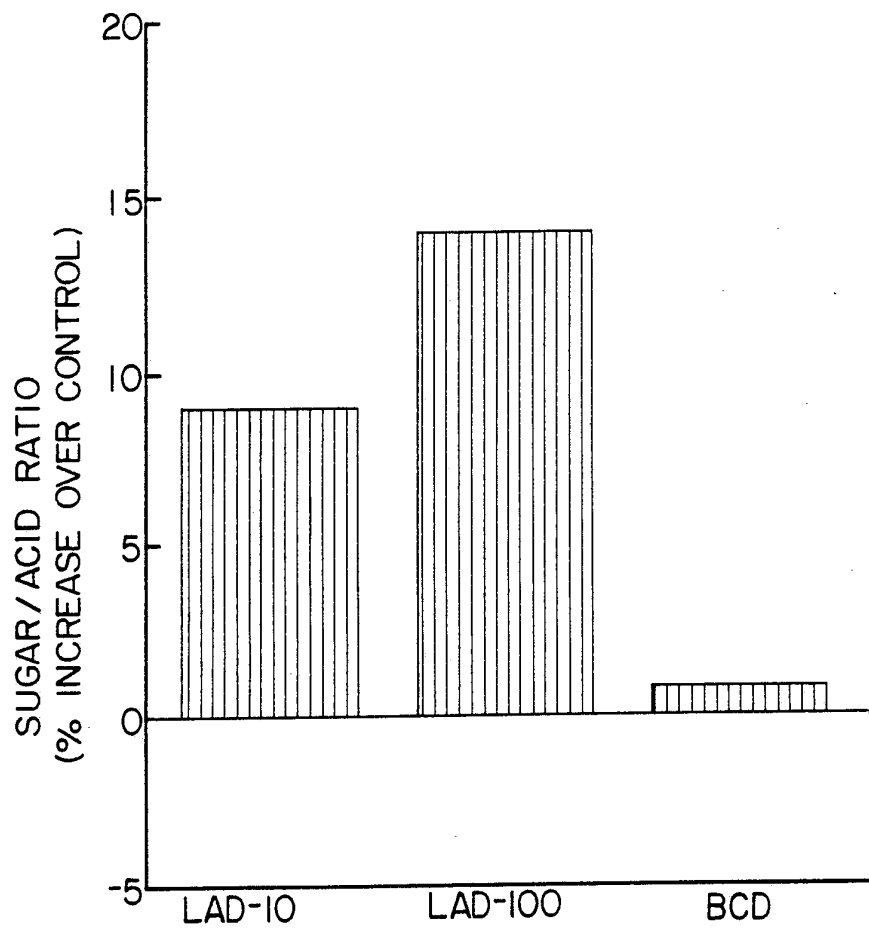


FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00134

A. CLASSIFICATION OF SUBJECT MATTER		
IPC(5) :A01N 43790 US CL :504/241 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols) U.S. : NONE		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) adenosine; ribofuranos-(w) adenine		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 2,881,164 (Kissman et al.) 07 April 1959 See entire document.	1-10
Y	US,A, 4,150,970 (Reis et al.) 24 April 1979 See columns 1-4.	1-10
Y	US,A, 4,333,758 (Welebir) 08 June 1982 See columns 1-4.	1-10
Y	US,A, 4,741,754 (Reis) 03 May 1988 See columns 1-3.	1-10
Y	Plant Physiology, 1991, Reis, "Triacontanol and Its Second Messenger 9-B-L(+)- Adenosine as Plant Growth Substances," 986-989. See entire document.	1-10
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be part of particular relevance *E* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed		*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *&* document member of the same patent family
Date of the actual completion of the international search 15 APRIL 1993		Date of mailing of the international search report 13 MAY 1993
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. NOT APPLICABLE		Authorized officer M.S. MARK CLARDY INTERNATIONAL DIVISION Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/00134

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 5,009,698 (Reis et al.), 23 April 1991. See columns 1-3.	1-10