The Fate of ^{14}C(2-Chloroethyl)phosphonic Acid in Summer Squash, Cucumber, and Tomato[1,2]

M. Yamaguchi, Celia Wang Chu, and S. F. Yang[3]
University of California, Davis

Abstract. Radioactive (2-chloroethyl)phosphonic acid (ethephon) was applied to leaves and fruits of 'Tiny Tim' tomato and 'Pioneer' cucumber and to seedlings of 'Yellow Crookneck' summer squash. During the first day, slightly over 21% of the applied ^{14}C-ethephon was converted to ^{14}C-ethylene by the squash plants, and 10 to 15% was converted by the tomato plants. A week after treatment the rate of ^{14}C-ethylene production decreased rapidly to less than 1% per day. Increases in rates of production of total ethylene following treatment were attributed to the decomposition of ethephon. Radioactive CO_2 production was small, amounting to about 0.1% of the ^{14}C applied.

Seven days following treatment of tomato leaves, about 15% of the ^{14}C was translocated to developing fruits and lesser amounts to other parts of the plant. In the squash seedling, from 3 to 9% was translocated after 2 days from the site of application to other tissues. Twenty-five days after application to cucumber leaves, the fruits contained only 0.3% of the applied ^{14}C-ethephon. In the tomato tissue the radioactivity was present as ^{14}C-ethephon, but in the squash seedling tissue much of the radioactivity was present in a new compound.

Numerous reports in the literature indicate that application of (2-chloroethyl)phosphonic acid (ethephon[4]) (1) to plants may induce various growth responses. These include changes in the initiation of female flowers of cucurbits (4, 9, 11, 13, 15–17, 19, 22) and accelerated ripening of many fruits (3, 5–8, 12, 18, 20, 21). The action of ethephon as a plant growth regulator has been attributed to a decomposition product, ethylene (1, 3, 6, 14, 19, 23, 24).

This paper reports the fate of ^{14}C-ethephon applied to fruits and leaves of tomato and cucumber plants and to leaves of summer squash seedlings.

Materials and Methods

Tomato. Dwarf tomato plants (*Lycopersicon esculentum* Mill. cv. Tiny Tim) were grown individually in 1-gal plastic containers in a greenhouse at Davis, California. When fruits were at the mature-green stage, 50 to 100 μl of a 200-ppm solution of ^{14}C-ethephon[5] (13.2 x 10^3 cpm/mμ mole) were applied as droplets to the surface of the fruits or to leaf blades. The treated plant was placed in a Pyrex jar 30 cm in diam and 60 cm high, through which water-saturated air flowed at the rate of 10 to 12 liters per hr. To collect the evolved ethylene (C_2H_4) and CO_2, the effluent gas was passed through 3 absorption tubes having sintered-glass diffuser systems. The first 2 tubes contained 50 ml of 0.25 M $Hg(ClO_4)_2$ in 2.0 M $HClO_4$ (25), and the third tube contained 25 ml of 10% KOH solution. The solutions were collected periodically and fresh solutions placed in the tubes. The 0 day rate of ethylene production was obtained by collecting the gas produced by the plant over a 24-hour period prior to application of ^{14}C-ethephon.

Squash. Summer squash seedlings (*Cucurbita pepo* L. cv. Yellow Crookneck) were grown, 2 to a pot, in the greenhouse during the late summer and also during the winter. At the 2-true-leaf stage (2 to 3 weeks after seeding), the plants were treated either by injecting 100 μl into each hollow petiole or by applying 50 μl as droplets to each leaf blade or growing point. The concn of ethephon (13.2 x 10^3 cpm/mμ mole) was 200 ppm in an aqueous medium. Immediately after the plants were treated, the pot was placed in a cylindrical Pyrex jar 20 cm in diam and 46 cm in height and the jar was sealed. Air which had been saturated with water was passed through the chamber at a rate of 3 to 6 liters per hr. Ethylene and CO_2 were collected as described above. Leaf samples were taken periodically, and the entire plant was analyzed for radioactivity at the conclusion of the experiment.

Cucumber. Seeds of a gynoecious cucumber (*Cucumis sativus* L. cv. Pioneer) were planted in the field early in June. Twenty-two days later, when plants were at the 4-true-leaf stage, 250 μl of a 400-ppm ^{14}C-ethephon. A field-grown 40-day-old plant having 6 very small fruits which had just set was used in this experiment. A total of 1.0 ml containing 10 x 10^6 cpm ^{14}C-ethephon (13.2 x 10^3 cpm/mμ mold) solution containing 8 x 10^6 were applied as droplets to each of the 4 leaves. At this time the first 2 leaves were fully expanded and the third and fourth leaves were still enlarging. There were no flowers on the plants. Twenty-two days after treatment of the leaves, one fruit was harvested; and after 25 days all the fruits on the vines and the 4 treated leaves were harvested. The remainder of the plant was not analyzed.

Fruits also were treated with ^{14}C-ethephon (13.2 x 10^3 cpm/mμ mole) was applied to 3 of the fruits. One of the treated fruits was harvested after 4 days. All the fruits, including the 3 additional fruits which developed after treatment, were harvested at termination of the experiment (7 days). Only the fruits were analyzed for radioactivity.

Except for the field experiments with cucumber, all greenhouse experiments were repeated at least twice and typical data are presented in the Results. The cucumber experiments could not be repeated because the supply of ^{14}C-ethephon was limited. These are included, however, to show that ethephon was translocated in this species.

Determination of radioactive CO_2, ethylene, and ethephon. Total ethylene absorbed in the $Hg(ClO_4)_2$ solution was determined as follows: a 5-ml vol of the solution was placed outside the center well in a 50-ml reaction flask, and the flask was then stoppered with a rubber serum cap. By means of a hypodermic syringe and needle, 5 ml of 2N HCl were added to the $Hg(ClO_4)_2$ solution. After 3 hr a sample of the atmosphere above the solution was withdrawn and the amount of ethylene was determined by gas chromatography.

To determine radioactive ethylene, 0.3 ml of 0.2 M $Hg(Ac)_2$ in methanol was injected into the center well of a 50 ml reaction flask. After 1 hr, the radioactivity of ethylene absorbed in the $Hg(Ac)_2$ solution was determined by liquid scintillation. Since $Hg(Ac)_2$ does not absorb the gaseous ethylene completely, a second determination of ethylene in the gaseous phase of the reaction flask was made by gas chromatography. A

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[3]Department of Vegetable Crops.

[4]Ethephon is the approved common name for (2-chloroethyl)phosphoric acid. ETHREL is the registered trademark of Amchem Products, Inc. for plant growth regulator formulations, the principal active ingredient of which is ethephon.

[5]^{14}C ethephon (CEPA-1,2-^{14}C) was a gift from Amchem Products, Inc. This compound gave a single peak on chromatograms resolved with several different solvent systems.

correction was made for the nonabsorbed ^{14}C–ethylene.

Radioactive CO_2 was determined in a manner similar to that described above for ethylene, except that 0.5 ml of ethanolamine–ethoxyethanol solution (1:1 by vol) was placed in the center well to absorb the $^{14}CO_2$ for liquid scintillation counting. One ml of 6N H_2SO_4 was injected to release CO_2 from the KOH solution.

Fruits or leaves which had been treated with ^{14}C–ethephon were rinsed twice with distilled water. The rinsing waters were combined and the radioactivity determined. The samples were weighted, then homogenized with 95% ethanol containing 1% formic acid. The homogenate was centrifuged at 27,000 x g for 20 min and the supernatant fluid was saved. The residue after centrifugation was extracted twice with 80% ethanol containing 1% formic acid. The alcohol extracts were combined and the radioactivity was determined.

Radioactive ethephon in the alcoholic extracts was determined as follows: The extract was first counted in a liquid scintillation counter without treatment; then the ethephon was decomposed at 60° for 8 hr with enough 20% KOH solution so that the final concentration of KOH was 7% in squash extracts and 10% in tomato extracts. After the alkali treatment, the sample was counted again, and the difference between the 2 counts was taken as the value for ^{14}C–ethephon.

Paper chromatography was used to identify the extracted ^{14}C compounds. Chromatograms (Whatman No. 1) were developed in butanol:HAc:H_2O (4:1:5 by volume). Radioactivity was monitored with a strip scanner.

Results

Tomato. There was a 2– to 3–fold increase in total ethylene production by the plant after application of ethephon to the fruits (Fig. 1). The increase in rate was continuous over the entire period of the experiment. Production of radioactive ethylene reached a maximum in 2 days, and by 5 days it was very low. The slow rise to the maximum was probably a reflection of the time required for the ^{14}C-ethephon to penetrate into the cell, where the pH was favorable for ethephon degradation. Alternatively, this time lag may also have occurred as a result of the large volume of the jar (38 liters) and the relatively slow airflow rate that were used. A more rapid flow rate was not used because it would have decreased the efficiency of ethylene absorption.

During the first 2 days after treatment the specific radioactivity of the evolved ethylene was slightly less than that of the ethephon which was applied. By the fifth day the specific activity was very low, which indicated that most of the ethylene production was not from decomposition of ethephon. Radioactive CO_2 amounted to 0.18% of the ^{14}C applied. Confirmation of $^{14}CO_2$ was by radio gas–chromatography as described by Baur and Yang (2).

About 65% of the activity applied was found on the surface of the fruits 1 day after application, and after 4 days the activity was down to 19% (Table 1). The amount of ^{14}C–ethephon in fruits which received the application was fairly constant throughout the experiment, ranging from 14 to 18% of the amount applied. There was translocation of radioactivity to other parts of the plant.

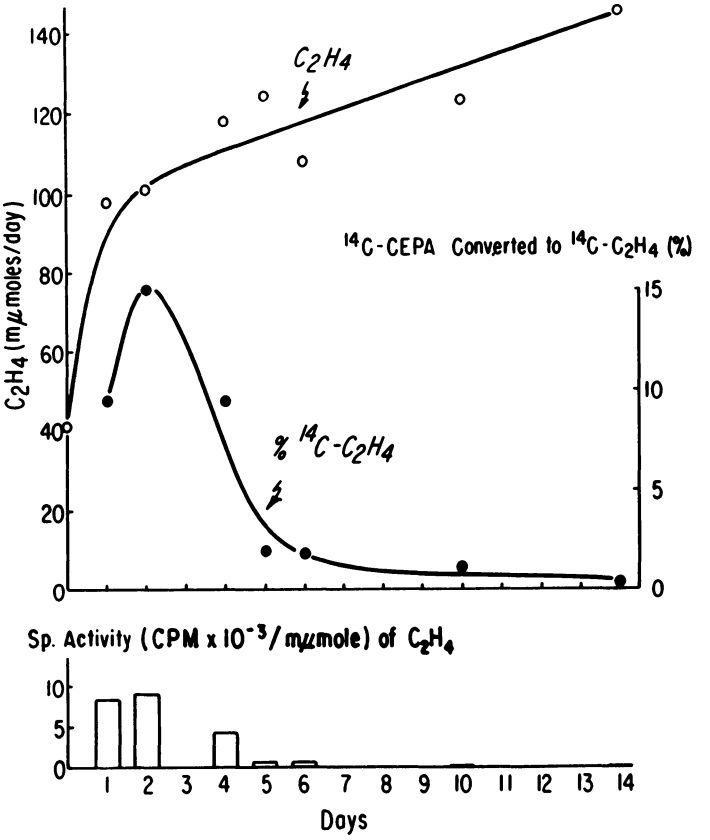

Fig. 1. Rate of total and radioactive ethylene production from tomato plants following the application of ^{14}C–ethephon to tomato fruits. Ethephon applied was 200 ppm and 648 mμmoles with a specific activity of 13.2 x 10^3 cpm/μmole. Total wt of plant including fruits was 143 g. CEPA = ethephon.

Table 1. Radioactivity of tomato fruits treated with ^{14}C-ethephon.[a]

Time after treatment (days)	Tissue sampled[b]	Fresh wt (g)	^{14}C-ethephon applied (cpm x 10^{-3})	Radioactivity			
				On surface[c]		In Tissue	
				(cpm x 10^{-3})	(%)[d]	(cpm x 10^{-3})	(%)[d]
1	Mature green	8.38	1745	1124	65	317	18
4	Breaker	7.51	1745	336	19	279	16
7	Ripe (red)	6.62	1570	102	7	217	14
12	Pink	12.87	1745	287	16	249	14
14	Ripe (red)	9.65	1745	e	e	303	17
14	Other fruits (green)	13.85	0	0	0	27	–
14	Rest of plant (leaves, stems, roots)	84.77	0	0	0	105	–
	Total		8550	1849		1497	–

[a]Total radioactivity in ethylene and CO_2 evolved during experiment = 3251 x 10^3 cpm. Recovery of ^{14}C = 77%.
[b]All fruits were mature green at time of application.
[c]Rinsed from fruit surface.
[d]Per cent of amount applied.
[e]Sample lost.

Results from applications of ^{14}C-ethephon to leaves of the tomato plant were similar to those obtained with applications to fruits. Figure 2 indicates the distribution of radioactivity in the plant after 7 days. About 15% of the radioactivity was still on the leaves which received the application and about 50% had been converted to ^{14}C–ethylene. The highest amount translocated (12%) was found in immature fruits on the same branch as the treated leaves. When the radioactive substance recovered from the fruit surface or from the tissue extracts was separated on paper, essentially all the radioactivity was found in the Rf zone of ethephon. That this substance was indeed ^{14}C–ethephon was further confirmed by degradation in alkali and radio gas chromatography, which yielded ^{14}C–ethylene. Therefore, we can conclude that the translocated compound was ethephon itself.

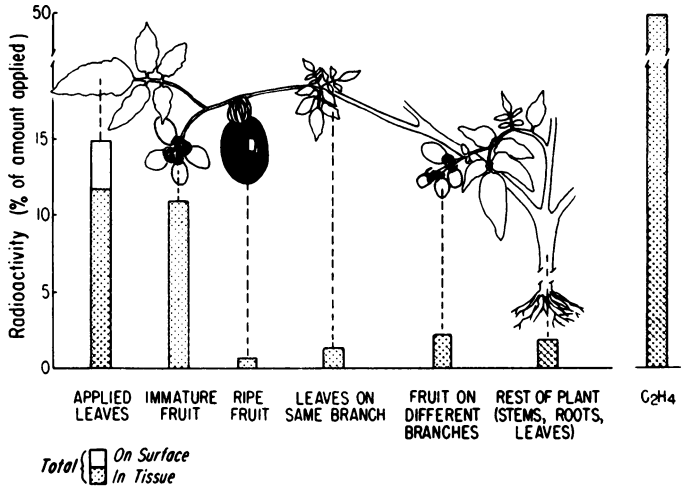

Fig. 2. Distribution of radioactivity in tomato plants 7 days after application of ^{14}C–ethephon leaves and amount of C_2H_4 produced.

Summer squash. When ethephon was injected into petioles the rate of production of total ethylene reached a peak during the first day, then rapidly declined to a low level after 3 days (Fig. 3). The rate of radioactive ethylene production followed a similar pattern. Over 20% of the applied ^{14}C–ethephon was converted to ethylene the first day, and slightly less than 15% the second day. From the specific radioactivity calculations, it appears that the burst of ethylene production by the seedlings during the first 2 days came principally from the applied ethephon and not from endogenous ethylene. The amount of $^{14}CO_2$ produced from the applied ^{14}C–ethephon was very low, amounting to less than 0.2% of the total ^{14}C applied.

The distribution of radioactivity in the plants which received petiole injections of ^{14}C–ethephon is indicated in Fig. 4. There was a rapid decline in radioactivity in the petioles after the first day. This was accompanied by translocation of radioactivity to other parts of the squash seedlings.

One day after application the radioactivity in the squash plant was mainly in ethephon. After 2 days, however, the presence of an unknown radioactive compound was noted (Fig. 5). After 6 days the radioactivity of the unknown metabolite at the site of application was greater than in ethephon. The translocated radio-activity was all in the unknown metabolite. Similar results were obtained after 14 days. Translocation of radioactivity as well as the formation of the unknown metabolite were also observed when ^{14}C–ethephon was applied as droplets to the leaf blade or to the growing point. The Rf of the unknown metabolite in the butanol–acetic acid–water system was 0.14, while that of ethephon was 0.45. With silicic acid thin-layer chromatography, using the same solvent system, the Rf of the unknown was 0.20 and that of ethephon was 0.63.

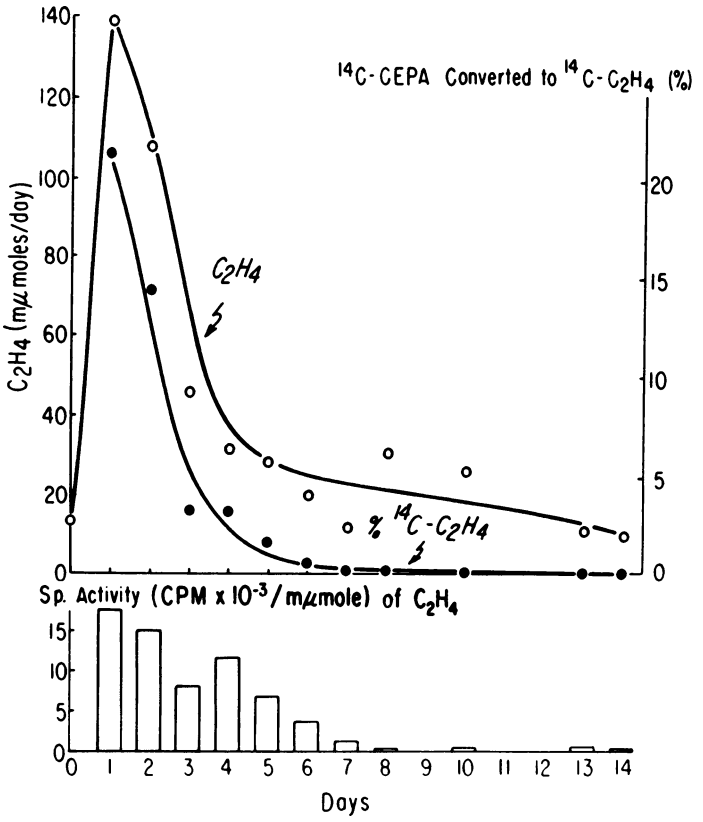

Fig. 3. Rate of total and radioactive ethylene production from summer squash following the application of ^{14}C–ethephon. Ethephon, 748 mμmoles, was applied as a 200–ppm solution with a specific activity of 13.2 x 10^3 cpm/mμmole. Total wt of plant was 143 g. CEPA = ethephon.

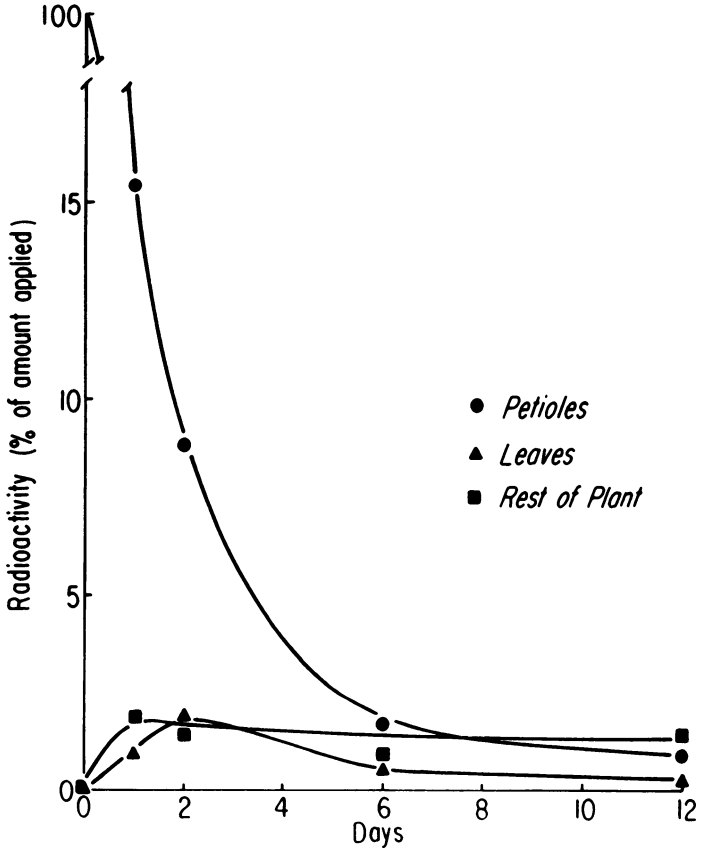

Fig. 4. Distribution of radioactivity in summer squash seedlings after application of ^{14}C-ethephon. A 200–ppm solution of ethephon containing 5720 x 10^3 cpm per plant was injected into petioles of plants at the 2–true–leaf stage.

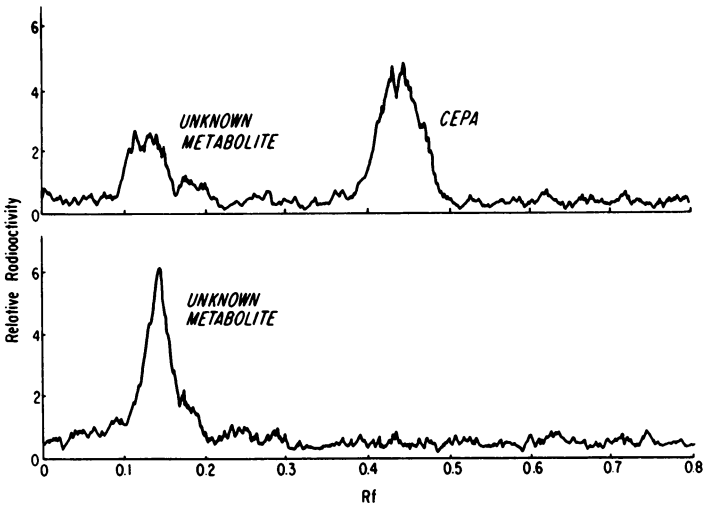

Fig. 5. Paper radiochromatograms of alcholic extracts of squash tissues developed in a solvent system of n-Butanol – acetic acid – water (4:1:5, by vol). *Top figure*. Two days after injection. Relative radioactivity vs. Rf of the extract of petiole tissue which was injected with ^{14}C–ethephon. *Bottom figure* – Six days after injection. Relative radioactivity vs. Rf of translocated compound in leaf blade tissue. CEPA = ethephon.

Cucumber. Only the treated leaves and fruits which developed following treatment were tested for radioactivity. The washings from the leaf surface and the alcohol extracts of the treated leaves contained ^{14}C–ethephon; this amounted to 11% of the total applied. The single fruit harvested 22 days after treatment weighed 26 g (no. 2 commercial size) and contained 0.1% of the applied ^{14}C. At termination of the experiment 25 days following leaf treatment, 3 of the large fruits, averaging 53 g (no.2 and no. 3's), contained 0.2% and the 14 small fruits, averaging 5 g (no. 1's) contained 0.1%. Because of the low radioactivity in the extract, we were unable to determine the Rf of the radioactive compound in the fruits.

It appeared that in fruits that had been treated with ethephon growth was almost completely arrested. Control fruits tagged at the same stage as the treated fruits had increased to about twice the initial length and diameter in 4 days, and had increased 3–fold in size by the 7th day. Four days following treatment about 40% of the total remaining radioactivity was found to be in ethephon. Translocation of radioactivity occurred to other fruits on the vine–to those fruits which had already set and also to fruits which subsequently developed. Radioactivity in these fruits amounted to less than 5% of the total applied. The radioactive compound in the latter fruits could not be identified because of low radioactivity.

Discussion

In the greenhouse, where experiments were conducted in a closed system, the recovery of radioactivity ranged from 77 to 91%. Losses of ethylene when the jar was opened periodically for sampling of tissues could account for part of the incomplete recovery.

On the basis of specific activity calculations, the initial increase in ethylene production in the tomato experiment was apparently due entirely to the release of ethylene from the applied ethephon. However, the increased production in the later stages of the experiment was probably the consequence of ripening of mature–green fruits. Dennis et al. (5) reported an initial increase of internal ethylene concentration in tomato fruits treated with ethephon. This was followed by a decline and then another increase on ripening.

The total amount of $^{14}CO_2$ collected amounted to less than 0.2% of the total radioactivity applied. This would indicate that little of the ethylene released from ethephon was metabolized and converted to CO_2 by the plants (10).

In the summer squash either the unknown metabolite was the more mobile form which was translocated preferentially from the petiole to other parts of the plant, or the ethephon was translocated first and then it was converted to the unknown metabolite. The present data are insufficient to determine which of the mechanisms was operative.

Continued translocation of ethephon or its metabolite occurred readily from the site of application in tomato, cucumber, and squash plants. Small but still measurable amounts of translocated radioactivity remained even after 14 days in tomato fruits and squash leaves and after 25 days in cucumber fruits. These attributes of ethephon, the movement of the compound in the plant and the slow degradation to ethylene, are important in explaining the effects, long after application, on tomato fruit ripening and sex expression of cucurbits.

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