The Fate of 1,2-^{14}C-(2-Chloroethyl)phosphonic Acid in Citrus[1]

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Abstract. ^{14}C-Ethylene was the major breakdown product of 1,2^{14}C-(2-chloroethyl)phosphonic acid (ethephon)-treated fruit and leaves of several *Citrus* taxons. Neither $^{14}CO_2$ nor other by-products were detected. Most of the nonethylene radioactivity recovered was from tissue surfaces. Radioactivity was not readily translocated from leaves or fruit.

The effects of (2-chloroethyl)phosphonic acid (ethephon) on fruit coloring (7, 8, 17, 19, 22) and abscission (5, 6, 8, 9) are well documented. Citrus fruit color has been reported to improve with ethephon sprays, and in most instances, some loosening has been reported (24–26). These responses appear to have been caused by ethylene, which originates from ethephon (20).

The absorption, translocation, and metabolism of ethephon have been studied in several plant types (1, 3, 10, 14–16, 21, 23), but not in citrus. This report summarizes studies with ^{14}C-ethephon on absorption, translocation, and metabolism in citrus tissues.

Materials and Methods

Plant material. Fruit or leaves on the following plants were used: 2-year-old budded trees of 'Satsuma' mandarin, *Citrus reticulata* Blanco; 'Robinson' tangerine, *C. reticulata* × (*C. paradisi* Macf. × *C. reticulata*), and 'Orlando' tangelo, *C. paradisi* × *C. reticulata*, grown in 5-gal containers; 10-year-old 'Lee' tangerine, *C. reticulata* × (*C. paradisi* × *C. reticulata*), trees grown under field conditions. In addition, detached fruit of 'Bearss' lemon, *C. limon* (L.) Burm. f., and 'Robinson' tangerines were used as test material. Two single-tree replicates were used for each treatment.

Field coloring test. 'Lee' tangerine trees were treated with ethephon to determine the relative effectiveness of site of application on color improvement and fruit-loosening. Two trees were sprayed with 300 ppm ethephon on October 5, 1973, with 10 fruit per tree bagged before spraying and remaining so until the trees were dry from the spray. Also, 10 fruit on each of 2 unsprayed trees were dipped in 300 ppm ethephon, with care taken not to contaminate adjacent stems and leaves. Fruit-rind chlorophyll and carotenoids were measured with a portable light-reflectance meter (13). Readings were taken before and 7 days after treatment, and the results were expressed as net changes. Fruit-loosening ratings were taken by measuring the force in kg required to separate the stem from the fruit.

^{14}C-Ethephon treatments. A stock solution of 1,000 ppm ^{14}C-ethephon (2.22×10^6 cpm/ml) was prepared with 0.1% Triton X-100 surfactant. Mature, green 'Bearss' lemon fruit were treated on August 31, 1972, with 200 μl 1,000 ppm ^{14}C-ethephon for a distribution study. Three single-fruit replicates were analyzed for total radioactivity 1, 4, 7, 11, and 14 days after treatment. On August 31, 1973, 2 fruit were treated with 100 μl 1,000 ppm ^{14}C-ethephon for an ethylene trapping study.

Leaves and fruit of each of 2 'Robinson' tangerine trees were treated with 100, 150, and 225 μl 333 ppm ^{14}C-ethephon (7.4×10^5 cpm/ml) on October 2, 1972, and tissues were harvested 5 days after treatment. One-third of each of 2 trees of 'Satsuma' mandarin and 'Orlando' tangelo were also sprayed with 333 ppm ^{14}C-ethephon on January 9, 1974, and samples were harvested 5, 10, 15, and 35 days after treatment. The remaining parts of the trees were shielded with plastic to avoid contamination from the spray.

^{14}C-Ethylene, $^{14}CO_2$ trapping tests. Treated, detached 'Bearss' lemon or 'Robinson' tangerine fruit were placed in a 1-qt plastic container, through which a gentle airstream passed. Evolved ethylene and CO_2 were collected by passing the effluent gas through 4 absorption tubes having sintered-glass diffuser systems. The first 3 tubes contained 25 ml of 0.25 M $Hg(ClO_4)_2$ in 2.0 M $HClO_4$ (23), and the fourth tube contained 25 ml of 10% KOH solution. The solutions were collected periodically and replaced with fresh ones.

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 of the center well in a 50-ml reaction flask, and the flask was stoppered with a rubber serum cap. By a hypodermic syringe, 5 ml of 2 N HCl was added to the $Hg(ClO_4)_2$ solution. After 24 hr, a sample of the atmosphere above the solution was withdrawn, and the amount of ethylene was determined by gas chromatography (18). Radioactive ethylene was determined by trapping in 0.3 ml of 0.2 M $Hg(Ac)_2$ in methanol that was in a center well of a 50-ml reaction flask. After 48 hr, the center well was removed, and the radioactivity was counted by liquid scintillation.

Radioactive CO_2 was determined in a similar manner, 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. To release CO_2 from the KOH solution recovered from the fourth absorption tube, 1 ml of 6 N H_2SO_4 was injected.

Fruit or leaves that had been treated with ^{14}C-ethephon were rinsed with 20% MeOH, and then radioactivity was determined. Tissue samples were homogenized with 80% ethanol containing 1% formic acid. The homogenate was centrifuged at 27,000 *g* for 20 min, and radioactivity was determined on the supernatant. Total radioactivity was determined by counting extract aliquots, and ^{14}C-ethephon was determined by treating extract aliquots with NaOH and determining the ^{14}C lost or by trapping ^{14}C-ethylene in 0.2 M $Hg(Ac)_2$.

Extracts were chromatographed on Gelman[3] I.T.L.C.-type SG chromatograph medium using N-butanol:HAc: H_2O (4:1:5) as the solvent system. The chromatogram was divided into 30 equal sections, and each section was placed in PPO:POPOP:toluene (0.5 g:5 g:1 liter) for counting. Radioactivity was determined with a Packard Tri Carb[3] scintillation spectrometer. Each sample was counted twice, to 10,000 counts or 100 min (whichever came first), and the counts were corrected for quenching. Samples with fewer than 2 counts above background are reported as zero.

Results

^{14}C-Ethylene evolution. Mature 'Bearss' lemon fruit treated with ^{14}C-ethephon evolved high levels of both total and ^{14}C-ethylene the first 2 days, and the evolution rates gradually declined thereafter up to 14 days (Fig. 1). The specific activity of ethylene was 185 to 240 cpm/nmole the first 6 days and 40 to 120 cpm/nmole between days 7 and 14. Similarly treated 'Robinson' tangerine fruit evolved the highest total and ^{14}C-ethylene the second day, with declining evolution rates thereafter (Fig. 2). Specific activity ranged from 92 to 138

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[3] Mention of a trademark name or a proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may also be suitable.
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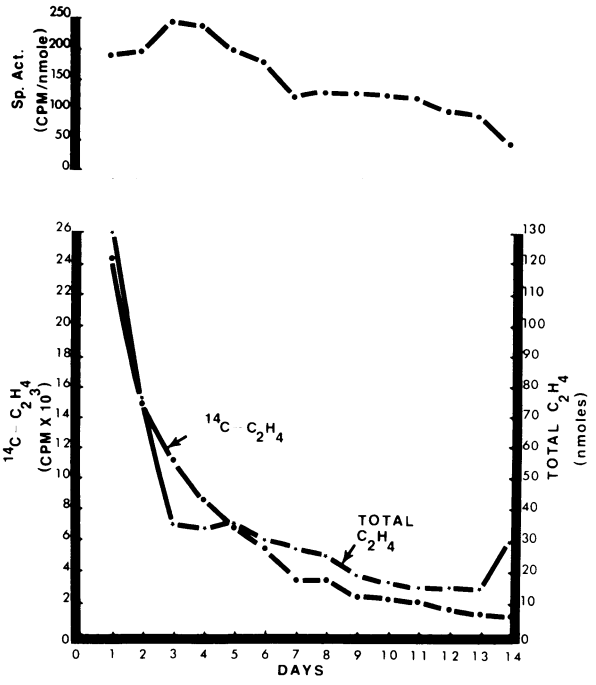

Fig. 1. Rate of total and radioactive ethylene produced by 'Bearss' lemon fruit after application of 100 μl 1,000 ppm ^{14}C-ethephon (2.2×10^6 cpm/ml).

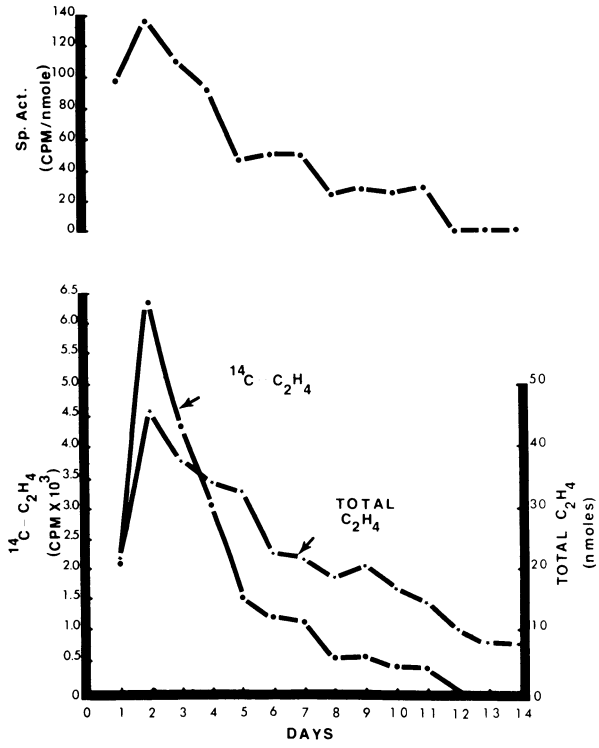

Fig. 2. Rate of total and radioactive ethylene produced from 'Robinson' tangerine fruit after application of 100 μl 333 ppm ^{14}C-ethephon (7.4×10^5 cpm/ml).

cpm/nmole the first 4 days and declined thereafter. No $^{14}CO_2$ was detected in the KOH trapping solution during the 14-day tests with either lemon or tangerine fruit.

^{14}C distribution in fruit. Total radioactivity of ^{14}C-ethephon-treated 'Bearss' lemon fruit decreased with time, and greatest activity was found on the fruit surface and in the rind (Table 1). There was little activity in the fruit segments. Total activity after 14 days was only 6.3% of the total applied, and most of that was on the rind surface. Some of the activity shown in the rind samples may have been trapped in the wax platelets on the fruit surface. Treated fruit were yellow after 7 days, whereas untreated fruit remained green.

Tree tests with ^{14}C-ethephon. 'Robinson' fruit terminal to treated leaves or on a shoot next to the treated leaves had little radioactivity (Table 2). There was some movement of radioactivity from the treated leaves to the subtending stems, but most of the activity was in the surface wash or in the leaf tissue.

Radioactivity from treated fruit did not move to fruit or leaves on adjacent shoots. Activity was confined mainly to the rind or rind surface of the treated fruit. Some activity did appear in an adjacent stem (Table 3). Internal atmosphere samples were taken from treated and untreated fruit, and radioactive ethylene was found only in the treated fruit.

Little radioactivity was translocated from the ^{14}C-ethephon sprayed 'Satsuma' mandarin tissues to untreated leaves and fruit, although considerable radioactivity was recovered from the treated tissues. Similar results were obtained with 'Orlando' tangelo (data not shown). When extracts of treated 'Satsuma' mandarin leaves, stems, fruit rind, and fruit segments were adjusted to 10% NaOH and heated for 8 hr at 60° C, all the radioactivity was lost.

Chromatographic separation of tissue extracts. Extracts of treated 'Bearss' lemon fruit rind and 'Robinson' tangerine fruit rind and leaves were concentrated by freeze drying and chromatographed. One peak of activity appeared in all samples, and the Rf corresponded to that of authentic ^{14}C-ethephon (Table 4). Little radioactivity was present on the remaining parts of the chromatograms.

Field coloring test. The coloring and loosening responses of 'Lee' tangerine fruit were evaluated at several ethephon application sites (Table 5). The best coloring response, which was measured by reduced chlorophyll and increased carotenoids, and fruit-loosening occurred when both leaves and fruit or the fruit alone were treated with

Table 1. Radioactivity from 'Bearss' lemon fruit after a postharvest ^{14}C-ethephon treatment.

Days after treatment	^{14}C Radioactivity recovered[z]				
	Surface wash	Rind	Segments	Total	Percent activity applied
	(cpm)	(cpm)	(cpm)	(cpm)	(%)
1	50,833	29,194	511	80,538	18.3
4	36,967	23,464	720	61,151	13.9
7	32,350	19,780	785	52,915	12.0
11	26,250	14,248	864	41,362	9.4
14	18,700	8,250	649	27,599	6.3

[z] 200 μl 1,000 ppm ^{14}C-ethephon (2.2×10^6 cpm/ml) was applied per fruit on August 31, 1972.

Table 2. Radioactivity from 'Robinson' tangerine fruit and shoots 5 days after leaves were treated with ^{14}C-ethephon.

Part analyzed	^{14}C Radioactivity recovered					
	Treated leaves[z]		Untreated fruit[y]		Treated shoot[x]	
	Total	Percent activity applied	Total	Percent activity applied	Total	Percent activity applied
	(cpm)	(%)	(cpm)	(%)	(cpm)	(%)
Surface wash	18,100	10.6	0	0.0	12,275	11.2
Leaves	11,094	6.5	—	—	8,319	7.6
Stem	2,742	1.6	0	0.0	0	0.0
Rind	—	—	0	0.0	0	0.0
Segments	—	—	0	0.0	0	0.0

[z] Leaves were treated with 225 μl 333 ppm ^{14}C-ethephon (7.4×10^5 cpm/ml) on October 2, 1972.
[y] Fruit were harvested from shoot next to treated shoot.
[x] Leaves were treated with 150 μl 333 ppm ^{14}C-ethephon (7.4×10^5 cpm/ml). Fruit were untreated and terminal to treated leaves.

Table 3. Radioactivity from 'Robinson' tangerine fruit and shoots 5 days after fruit were treated with ^{14}C-ethephon.

Part analyzed	^{14}C Radioactivity recovered					
	Treated fruit[z]		Untreated fruit[y]		Untreated leaves[y]	
	Total	Percent activity applied	Total	Percent activity applied	Total	Percent activity applied
	(cpm)	(%)	(cpm)	(%)	(cpm)	(%)
Surface wash	13,000	17.6	0	0.0	0	0.0
Rind	3,072	4.2	0	0.0	—	—
Segments	0	0.0	0	0.0	—	—
Stem	1,156	1.6	1,175	1.6	0	0.0
Leaves	—	—	—	—	—	0.0

[z] Fruit were treated with 100 μl 333 ppm ^{14}C-ethephon (7.4×10^5 cpm/ml) on October 2, 1972.
[y] Fruit and leaves were on adjacent shoots.

Table 4. Radioactivity of bands of separated ^{14}C-ethephon treated tissue extracts from paper chromatograms.

Sample and time from application	Location on chromatogram	Total activity on chromatogram
	(Rf)	(cpm)
Authentic ^{14}C-ethephon	0.83–0.93	22,979
Bearss lemon fruit rind:[z]		
1 day	0.83–0.93	710
7 days	0.83–0.93	760
14 days	0.83–0.93	263
Robinson tangerine fruit rind:[y]		
5 days	0.83–0.93	38
Robinson tangerine leaves:[x]		
5 days	0.83–0.93	113

[z] Aliquots of extracts shown in Table 1.
[y] Aliquots of rind extract shown in Table 3.
[x] Aliquots of leaf extracts shown in Table 2.

Table 5. 'Lee' tangerine fruit loosening and coloring response[z] from 300 ppm ethephon application.

Treatment	Fruit loosening response		Coloring response	
	Pull force	Plugging[y]	Chlorophyll	Carotenoids
	(kg)	(%)	($\Delta\Delta$R)	($\Delta\Delta$R)
Control	3.9	100	−0.04	+0.06
Spray leaves and fruit	2.2	10	−0.12	+0.17
Spray leaves only	3.7	70	+0.01	+0.03
Dip fruit only	1.3	5	−0.18	+0.28

[z] Treatments applied on October 5, 1973.
[y] Hole left in rind when the fruit was pulled from the stem.

ethephon. Treating only the leaves did not induce changes in color or loosen the fruit.

Discussion

'Bearss' lemon and 'Robinson' tangerine fruit treated with ^{14}C-ethephon evolved radioactive ethylene with a high specific activity. This result showed that ethephon rather than the tissue itself, was the major source of ethylene in ethephon-treated citrus fruit. ^{14}C-ethylene has been shown to be a by-product of ^{14}C-ethephon with ethephon-treated cherry leaves (10) and tomato fruit (23), but the tomatoes stimulated by ethephon also produced endogenous non-labeled ethylene. Others assumed that the loss of radioactivity with time from ^{14}C-ethephon treatment was due to evolved ^{14}C-ethylene (1, 10, 14–16, 21). Because we detected no $^{14}CO_2$ produced from the ^{14}C-ethephon-treated fruit up to 14 days after treatment, we concluded that ethephon C atoms were not metabolized in the usual respiratory cycle. This conclusion was strengthened by the facts that a) chromatographed ^{14}C-ethephon-treated tissue extract had 1 radioactive area corresponding to ^{14}C-ethephon and b) virtually all ^{14}C activity in the extracts was removed as ^{14}C-ethylene at 60° C by treatment with 10% base, which degrades parent ^{14}C-ethephon to ethylene and salts. Tomato (23), walnut (16), grape (21), apple, and cherries (10) have been reported not to metabolize ethephon (other than ethylene release). However, unknown metabolic products have been reported in ethephon-treated squash (23), peach (15), and *Hevea brasiliensis* tissues (3). Many plants (2), including citrus (4, 11), do not use ethylene as a C source efficiently. Our results suggest that ethylene from ethephon, at the concentration and exposure times used, was not metabolized in any quantity by citrus leaves and fruit.

Ethephon was not translocated rapidly throughout the citrus plant, but remained near the site of application. Small amounts of ethephon were translocated from leaves or fruit into subtending stems, but the distance moved was no more than 5 to 10 cm. Ethephon was translocated more freely in other crops (10, 16, 21), particularly tomato and squash (23). The lack of significant translocation in citrus relates to our findings with 'Lee' tangerine in this study: to get good color or abscission responses, the fruit, rather than the leaves, must be treated. Similar color responses were obtained with the same ethephon-treatment sites on 'Bearss' lemons (12). Thus, complete fruit coverage is essential with citrus to obtain good color, or abscission responses with ethephon.

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Minimum Irrigation Requirements for Landscape Plants[1]

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Abstract. Several field-established broadleaved and coniferous evergreen shrubs and 2 ground covers, *Carpobrotus* sp. and *Hedera helix* L., survived, and maintained adequate appearance with greatly restricted growth, without supplemental irrigation from May through September on deep soils at San Jose and Santa Ana, CA. *Eugenia uniflora* L. at Santa Ana required 1 irrigation (9.4 cm) in July to insure survival and both *Coprosma baueri* Endl. and *Cotoneaster pannosa* Franch. required 1 or 2 irrigations to insure adequate foliar density. At San Jose only *Nerium oleander* L. lost leaves or lost leaf color and turgidity in the non-irrigated plot. The plantings at both locations had viable roots down to 1 m and probably deeper. Non-irrigated and bimonthly irrigated soils were at or below the permanent wilting percentage down to 1 m. Leaf temp in the non-irrigated *Xylosma congestum* (Lour.) Merr. and *Carpobrotus* plots were 6 and 15° C, respectively, above ambient and yet no permanent foliar injury was observed. We suggest that leaf temp may be used to measure critical water stress in landscape plants. Our findings indicate that substantial savings in water costs and in controlling vegetative overgrowth can be realized by reducing irrigation frequency in established landscape plantings.

In many crop plants there is a direct, although not linear, correlation between consumptive water use and yield (6, 9, 11, 14). That is, maximum yields per unit land area are attained generally with high irrigation frequency to prevent water stress (11, 14) although Shmeuli (13) presents a more complex view of optimization of irrigation for each crop. For landscape plants yield is not a factor, only appearance and, ultimately, survival. We wish to maintain the minimum leaf area consistent with acceptable appearance and shading or screening functions. Thus, irrigation requirements for established landscape plants should be quite different from and, overall, considerably less than for comparable areas of crop species. Nevertheless, in many areas of California deep and shallow irrigations, particularly for species indigenous to humid regions, are made monthly, or even more frequently in warm weather from May through September.

We undertook research at the San Jose and Santa Ana (South Coast) Field Stations to determine the influence of reduced irrigation schedules on the growth, appearance, and survival of several commonly planted evergreen shrubs, trees, and ground covers.

Materials and Methods

Three irrigation regimes (Table 1) were established: a) biweekly at San Jose and monthly at Santa Ana, the normal schedule for orchard plantings at both locations; b) bi-monthly; c) no irrigation. Furrow irrigation was employed. At each irrigation approximately 9.4 cm was applied; the furrows were flooded for 8 hours and the soil was wet to an approximate depth of 65 cm. The rainfall and irrigation schedule for 1974 at San Jose and at Santa Ana are shown in Table 1. In the years prior to testing, when the plants were established, the blocks were irrigated at 30-day intervals and fertilized with complete nutrients once annually. There were 4 plants per treatment for *Xylosma*, *Oleander*, *Cotoneaster*, *Juniper*, *Eugenia*, and *Coprosma* and 5 plants per treatment for *Hedera* and *Carpobrotus*. At both Santa Ana and San Jose the woody perennial plantings were at least 4 years old and growing uniformly. The ice plant (*Carpobrotus* sp) and English ivy (*Hedera helix* L.) plantings at San Jose were entering their second year at the start of the irrigation treatments. Irrigation commenced at both locations approximately 30 days after the last substantial rainfall (>0.25 cm) in the spring.

Soil samples, to determine moisture content, were taken at the end of the experimental period using a soil auger; they were placed in cans with tight-fitting lids. The soil samples were oven dried (100° C) for 24 hours and gravimetric moisture content was calculated.

Plant growth was estimated by branch elongation of 10 randomly selected branches for a 60 cm^2 area of each block of 4 plants.

Leaf temp were estimated with an infra-red pistol thermometer (Raytek Model LR-120/n). Emissivity of all species was assumed to be 1.

Results and Discussion

Growth, whatever the parameter used, was closely correlated with irrigation frequency in all species examined (Figs. 1 and 2). At Santa Ana differences in growth rate (Fig. 3, lower) were apparent 30 days after the last rainfall or irrigation. There were sharp increases in

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