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

Hesham A. Abdel-Gawad[2] and George C. Martin
*University of California, Davis*

*Abstract.* **Forty days after treatment with $^{14}$C-ethephon 23.6% of the applied radioactivity was recovered from the total fruit. The level was reduced to 19.0% after 95 days as a result of lower radioactivity in the extracted fruit; the surface level remained constant. Radioactive metabolites noted by 40 days after treatment were still prominent by 95 days.**

Ethephon releases ethylene upon breakdown (11, 14), and the ethylene produced has a role in the regulation of plant growth (3, 10). Applied ethephon stimulates abscission of cotton leaves (8), fruits of cherries and plums (2), and walnuts (6). In addition, ethephon appears promising as a thinner of peach fruits when applied between full bloom and "June drop" (1, 4, 9).

The fate and movement of ethephon have also been studied. Yamaguchi et al. (13) found no metabolite of $^{14}$C-ethephon in treated tomato fruits. In squash, however, an unknown metabolite appeared in the fruits within 2 days after treatment, and the $^{14}$C-ethephon was largely converted to the new metabolite within 6 days. In contrast, Weaver et al. (12) and Martin et al. (7) found no metabolite of $^{14}$C-ethephon in grapes or walnuts in a 7-day period immediately after treatment. Edgerton and Hatch (5), also, reported the absence of metabolite of $^{14}$C-ethephon in fruits of apples and cherries.

In the present study, we investigated the fate of ethephon after application to young peach fruits.

*$^{14}$C-ethephon application.* A mature peach tree (*Prunus persica* cv. Halford) growing in the University of California experimental orchard at Davis, was used in this study. Branches were tagged, and the fruits were thinned to 1 peach of uniform size per branch. A band of bark, 1 cm wide, was removed from the base of each branch. On May 30, 1971, during cytokinesis, the branches were sprayed to the point of drip with 5 ppm unlabeled ethephon in 0.1% X-77 wetting agent. One hr later, 1 μc of $^{14}$C-ethephon was applied with a microsyringe to the surface of each fruit. Another μc was applied to the lower surface of the leaf immediately basipetal to the treated fruit. Ten fruits were initially treated but only 4 remained on the tree beyond "June drop." Two of these were taken at 40 days and the other 2 at 95 days posttreatment.

*Preparation of tissue and extraction.* The fruits were washed with methanol, freeze-dried, and then stored at −20°C until used. The washings were saved for later assay. Prior to extraction, individual freeze-dried fruits were ground with mortar and pestle under liquid nitrogen and quantitatively transferred to a plastic centrifuge tube. The ground fruit samples were added to 25 ml absolute methanol, pH 1.0, containing 250 ppm non-radioactive ethephon, and placed on a mechanical shaker for 48 hr to extract the radioactive material. The mixture was then centrifuged for 30 min at 17,300 × g. The supernatant fluid was decanted, and the pellet was resuspended in 15 ml absolute methanol and reextracted 4 times for 48 hr each. The extracts were combined, yielding a total of 100 ml.

*Determination of total $^{14}$C activity.* We transferred 1 ml of the methanolic extract to each of several plastic scintillation vials, and 19 ml of a scintillation fluid consisting of 500 ml 1,4-dioxane, 100 ml 2-ethoxyethanol (cellosolve), 6.0 g PPO, 0.3 g POPOP, and 30.0 g naphthalene were added. The vials were then placed in a Packard Tri Carb liquid scintillation spectrometer and counted at least 3 times for 10 min each (7).

*Correction for quenching.* Methanol, a good extraction solvent, is also a strong quenching agent. Pigments extracted from the fruits also contribute to quenching. To correct for quenching, each vial was carefully opened after $^{14}$C radioactivity was determined a known amount of $^{14}$C-ethephon was added, and the vials were closed, shaken, and counted again. Values which appear in the tables are corrected values.

*Metabolic studies.* A portion of the methanolic extract of the fruit samples was concentrated to dryness under vacuum at 30°C, and the residue dissolved in 5 ml absolute methanol. Fifteen μl of this concentrate were spotted on thin-layer chromatographic Avicel plates having an emulsion 250 μm thick (purchased from Analtech, Inc., Newark, Del.). A standard containing 5000 cpm $^{14}$C-ethephon was spotted on samples and adjacent to samples. All TLC plates were developed from the origin to the 10-cm mark in either a) benzene, concentrated acetic acid, and water (8:3:5, v/v/v), or b) methanol, isopropanol, concentrated ammonium hydroxide, and water (9:6:2:3, v/v/v/v). A TLC scanner (Actinograph III, Nuclear-Chicago) was used to locate radioactivity on the developed plates. The plates were then placed against X-ray film for 8 weeks to obtain an autoradiograph of the radioactive zones.

*Total $^{14}$C radioactivity recovered.* Forty days after treatment, 23.6% of the applied radioactivity was recovered from the total fruit (Table 1), compared to 19.0% recovered after 95 days. The decrease in the total radioactivity at 95 days posttreatment was the result of the marked decline in radioactivity in the extracted fruit after surface washing. The radioactivity washed from the surface of the peach fruits 40 days after treatment was 16.7% of that originally applied to leaves and fruits. At 95 days posttreatment, the value was 17.9% (Table 1). The radioactivity in the fruit flesh at 40 days decreased from 6.9% of that originally applied to 1.06% at 95 days posttreatment. The lower percent radioactivity in the fruit flesh at 95 days may have resulted from the metabolism of ethephon. The sustained higher level of radioactivity on the fruit surface may have been due to a slower rate of breakdown than in the flesh. It also indicates that penetration through the

*Table 1.* Radioactivity recovered from $^{14}$C-ethephon-treated peach fruits. Two μc of $^{14}$C-ethephon were applied per treatment, 1 μc to the fruit and 1 μc to a leaf immediately basipetal to the treated fruit. Each treatment value represents an average of 2 samples.

| Days after treatment | Method of recovery | $^{14}$C radioactivity recovered | |
|---|---|---|---|
| | | cpm x $10^3$ | Percent of total applied $^{14}$C-ethephon |
| 40 | Surface wash by methanol | 884.8 | 16.7 |
| | Extracted by methanol | 351.4 | 6.9 |
| | Total | 1,236 | 23.6 |
| 95 | Surface wash by methanol | 948 | 17.90 |
| | Extracted by methanol | 56.4 | 1.06 |
| | Total | 1,004 | 19.0 |

[1]Received for publication January 2, 1973.
[2]Present address: Department of Botany.

exocarp is insignificant and that weathering has not occurred.

*Metabolic studies.* Radiochromatographic scans of methanolic extracts of fruits revealed no evidence of any $^{14}C$ metabolite. All radioactivity in the samples occurred at the Rf of $^{14}C$-ethephon. However, an autoradiogram of the $^{14}C$ active peaks (Fig. 1 and 2) revealed metabolites in fruits harvested 40 days after treatment which were still evident by 95 days after treatment.

The extracts which were streaked on the TLC plates were quite viscous, particularly those at 95 days. As a result, the solvent moved through the origin more slowly than was the case with the $^{14}C$-ethephon standard. This affected the ultimate Rf of each sample compared to the standard.

Research has been initiated with the intention of identifying the unknown metabolites noted in this study.

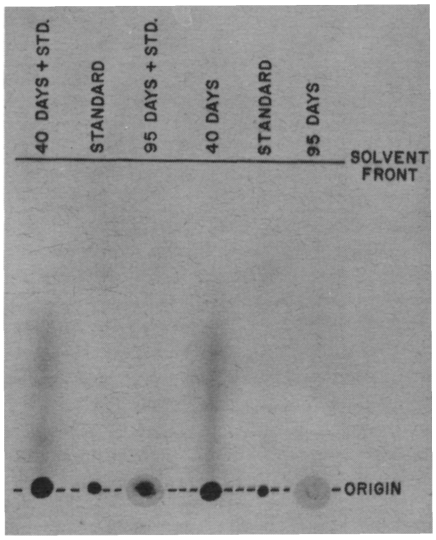


*Fig. 1.* Autoradiograph of extracts of peach fruits treated with $^{14}C$-ethephon. Samples were taken 40 and 95 days after treatment. TLC plates were developed in benzene:acetic acid:water (8:3:5, v/v/v).

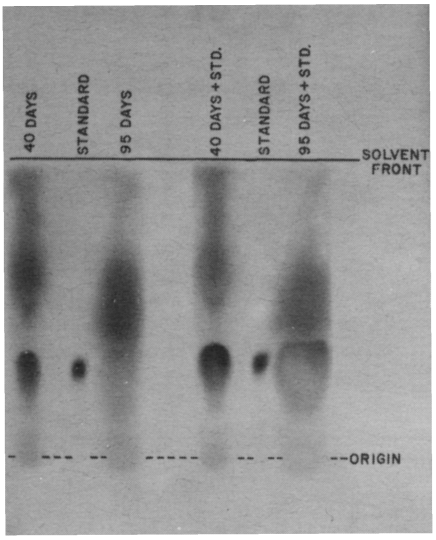


*Fig. 2.* Autoradiograph of extracts of peach fruits treated with $^{14}C$-ethephon. Samples were taken 40 and 95 days after treatment. TLC plates were developed in methanol:isopropanol:ammonium hydroxide:water (9:6:2:3, v/v/v/v).

### Literature Cited


1. Buchanan, D. W., and R. H. Biggs. 1969. Peach fruit abscission and pollen germination as influenced by ethylene and 2-chloroethanephosphonic acid. *J. Amer. Soc. Hort. Sci.* 94:327-329.
2. Bukovac, M. J., F. Zucconi, R. P. Larsen, and C. D. Kesner. 1969. Chemical promotion of fruit abscission in cherries and plums with special reference to 2-chloroethylphosphonic acid. *J. Amer. Soc. Hort. Sci.* 94:226-230.
3. Edgerton, L. J., and G. D. Blanpied. 1968. Regulation of growth and fruit maturation with 2-chloroethanephosphonic acid. *Nature* 219:1064-1065.
4. _____, and W. J. Greenhalgh. 1969. Regulation of growth, flowering and fruit abscission with 2-chloroethanephosphonic acid. *J. Amer. Soc. Hort. Sci.* 94:11-13.
5. _____, and A. H. Hatch. 1972. Absorption and metabolism of $^{14}C$(2-chloroethyl)phosphonic acid in apples and cherries. *J. Amer. Soc. Hort. Sci.* 97:112-115.
6. Martin, George C. 1971. 2-chloroethylphosphonic acid as an aid to mechanical harvesting of English walnuts. *J. Amer. Soc. Hort. Sci.* 94:434-436.
7. _____, H. A. Abdel-Gawad, and R. J. Weaver. 1972. The movement and fate of 2-chloroethylphosphonic acid in walnut. *J. Amer. Soc. Hort. Sci.* 97:51-54.
8. Morgan, P. W. 1969. Stimulation of ethylene evolution and abscission in cotton by 2-chloroethanephosphonic acid. *Plant Physiol.* 44:337-341.
9. Stembridge, G. E., and C. E. Gambrell, Jr. 1971. Thinning peaches with bloom and postbloom applications of 2-chloroethylphosphonic acid. *J. Amer. Soc. Hort. Sci.* 96:7-10.
10. Warner, H. L., and A. C. Leopold. 1967. Plant growth regulation by stimulation of ethylene production. *BioScience* 17:722.
11. _____, and _____. 1969. Ethylene evolution from 2-chloroethylphosphonic acid. *Plant Physiol.* 45:156-158.
12. Weaver, R. J., H. A. Abdel-Gawad, and George C. Martin. 1972. Translocation and persistence of 1,2-$^{14}C$-(2-chloroethyl)phosphonic acid (ethephon) in Thompson seedless grapes. *Physiol. Plant.* 26:13-16.
13. Yamaguchi, M., C. W. Chu, and S. F. Yang. 1971. The fate of $^{14}C$ (2-chloroethyl)phosphonic acid in summer squash, cucumber, and tomato. *J. Amer. Soc. Hort. Sci.* 96:606-609.
14. Yang, S. F. 1969. Ethylene evolution from 2-chloroethylphosphonic acid. *Plant Physiol.* 44:1203-1204.


# Boron Levels in Stems, Leaves, and Flower Parts of Carnation[1]


Gretchen A. Heuterman, C. G. Woodbridge and Elwood W. Kalin[2]
*Washington State University, Pullman*



*Abstract.* **The B levels of green tissues of *Dianthus caryophyllus* L. cv. Improved White Sim grown in sand culture increased but those of the important reproductive parts did not vary as solution B concentration increased over a range of adequate levels.**


Symptoms of B deficiency in carnations have been reported by Mastalerz et al. (5), Nelson and Boodley (7) and Oertli (8) but there have been some differences as to which tissues were first affected and their severity. There is limited information on the B levels of tissues other than the leaves recommended for foliar analysis purposes. In this study B levels in the stems, leaves and separated flower parts of plants are noted and symptomatology of B deficiency in carnation is described.

We planted 240 rooted cuttings in July 1969 in 4 replicates, each of 10 plants. Hoagland's No. 1 solution (3), with minor elements was used, but the concn of B, as boric acid, was varied (Table 1). Plant tissues were collected as the flowers reached senescence. Two crops from the same plants were collected and analyzed separately. The stems and leaves were separated from the flower which was divided into petals, stigma-style, ovary contents (placenta and ovules), ovary wall, calyx, and receptacle.

The tissues were analyzed for B by either the quinalizarin (6) or curcumin (1) method depending upon sample size. Since there were no differences (5% level) between the B levels of the parts from the 2 sampling dates, the data were combined (Table 1).

The first indication of deficiency was a shortening of the internodes below the bud 7 months after the plants had been planted (Fig. 1). At this time the tips of the leaves, directly below the bud, turned reddish purple. The stems became stiff and it was difficult to disbud the blooms as the side buds did not snap as readily as in those plants receiving B. The buds ceased to grow, became purplish at the tip, and dried to a straw color. A decrease in the no. of petals (Fig. 2), a prominent stigma-style and epinastic curvature of the bud as previously reported (5) were noted.


[1]Received for publication December 6, 1972. Scientific Paper No. 3966. Washington Agricultural Experiment Station, Pullman. Project No. 0049.


[2]Respectively: Graduate student, Professor of Horticulture and Professor of Floriculture, Department of Horticulture.