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Ethephon [1,2-^{14}C(2-chloroethyl)phosphonic acid] in Peach Fruits. I. Penetration and Persistance[1]

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Abstract. Penetration of ethephon into peach fruits was determined using 1,2-^{14}C(2-chloroethyl)-phosphonic acid (^{14}C-CEPA). Most of the activity accumulated in the fruit exocarp, with only minute amounts in the mesocarp. No notable lateral translocation was apparent. The results suggest that active degradation of the compound takes place in the young fruit while in the older peach fruits the rate of ^{14}C-CEPA degradation takes place at a slower rate.

The effects of 2-chloroethyl phosphonic acid (CEPA) on ripening (3, 5, 7, 13) and abscission (2, 5, 8, 10) of fruits is well known. In many species, treatments with commercial ethephon are common practice (9, 11). Peaches are relatively sensitive to this regulator, with enhanced coloration (14) and fruit drop occurring at low concn. Treatment at the 8-10 mm seed length stage is effective for thinning peach fruits (14). The rate of uptake and stability of CEPA, as well as its movement in the peach fruit, are only partially known (1). In this study, surface decomposition, penetration, and translocation in the fruit were studied using ^{14}C-labeled CEPA.

Materials and Methods

The fruits on a 13-year-old 'Halford' peach tree (*Prunus persica* L.) in the University of California experimental orchard were hand thinned to 1 per branch 18"-24" long shortly after fruit set. Full bloom occurred on March 7, 1972 and developing fruits were treated on May 15 and a second set on June 23, 1972 with ^{14}C-CEPA in methanol (specific activity 4.1 mCi/mM). One-hundred μl of ^{14}C-CEPA (total activity 2 μCi) were applied to one side of each treated fruit. All 7 fruit treated on June 23 were taken 65 days post-treatment for use individually in testing purification procedures and analysis of recoverable ^{14}C activity. Unwashed and water washed (running tap water) exocarp and 2 mesocarp layers, each 1.5 mm thick, from underneath the treated exocarp, were separately macerated in 1% HCl in methanol, then extracted 3 times for 30 min. The methanolic extracts were combined, and the solution was partitioned against petroleum ether (boiling point 30-60^{o}C). The methanol layer was then concd to the aqueous phase which was partitioned against petroleum ether. The aqueous fraction was further reduced to a minimum, avoiding dryness, and then was adjusted with 100% methanol to 1 ml/g fr wt of tissue. Twenty μl aliquots of these solutions were applied to Whatmann 3 MM paper (9 x 15 mm) and assayed in 1 ml toluene containing 5 mg PPO and 0.2 mg POPOP, using a Packard Tricarb scintillation counter with a counting efficiency of about 80%. Ten μl aliquots were applied to thin layer Gelman ITLC-SAF plates and developed with butanol:acetic acid:water (40:11:29 v/v/v) or isopropanol:ammonia:water (8:1:1 v/v/v), in an ascending chromatographic system. The radioactivity on

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the developed chromatograms was counted with a Nuclear Chicago strip counter.

Fruit treated on May 15 were sampled 21, 45, 70 and 95 days post-treatment taking 4 fruit at each date. Extraction procedures were followed as described above for individual fruit. Analysis of the mesocarp was accomplished with a 2 mm layer below the exocarp. On each collection date a separate sample of fruits were washed, halved and the endocarp removed. The halved fruits were pressed onto Whatmann 3 MM paper or a semi-absorbing paper. The resulting fruit prints were rapidly dried under a stream of hot air and used for autoradiography with Kodak single-coated, medical x-ray film. In some instances, thin slices of the fruits were dried and autoradiographed directly. An exposure of 21-25 days was optimal for our conditions.

Table 1. Distribution of radioactivity in ^{14}C-CEPA-treated 'Halford' fruits. (Treated June 23, 1972; harvested August 28, 1972).

Fruit part	Radioactivity (cpm/g fr wt) Treated side	Non-treated side
Unwashed exocarp	73210	760
Washed exocarp	22300[y]	840
Mesocarp I[z]	940	678
Mesocarp II[y]	410	420

[z]Mesocarp I - 1.5 mm mesocarp layer immediately below exocarp.

[y]Mesocarp II - 1.5 mm mesocarp layer immediately below the first layer.

Results and Discussion

Less than 1.8% of the radioactivity applied was recovered from the entire fruits 65 days after treatment (Table 1). A considerable amount of radioactivity was still present on the exocarp. A large portion of this could be washed off by a stream of cold water and very little penetrated into the exocarp and mesocarp. Progressively smaller amounts of radioactivity were found in the 2 mesocarp samples taken from beneath the exocarp. Relative to the total level of radioactivity, little translocation was detected, and significant activity was found only at the treated areas. The low level of activity that was present on the untreated side of the fruits might have been due to contamination arising from handling of the fruits. After extraction, only 1% of the activity of the exocarp and 2% of that in the mesocarp was left in the residue. The distribution or radioactivity in the fruits is further evident in autoradiograms of imprints of the cut surfaces of halved fruits on absorbing paper (Fig. 1).

Following chromatography of the extracts, radioactivity was found at the Rf of standard ^{14}C-CEPA. Chromatography in both solvent systems demonstrated that most of the radioactivity was due to unchanged ^{14}C-CEPA (Fig. 2). Division of the chromatograms into 10 equal segments followed by scintillation counting indicated that 95-98% of the total

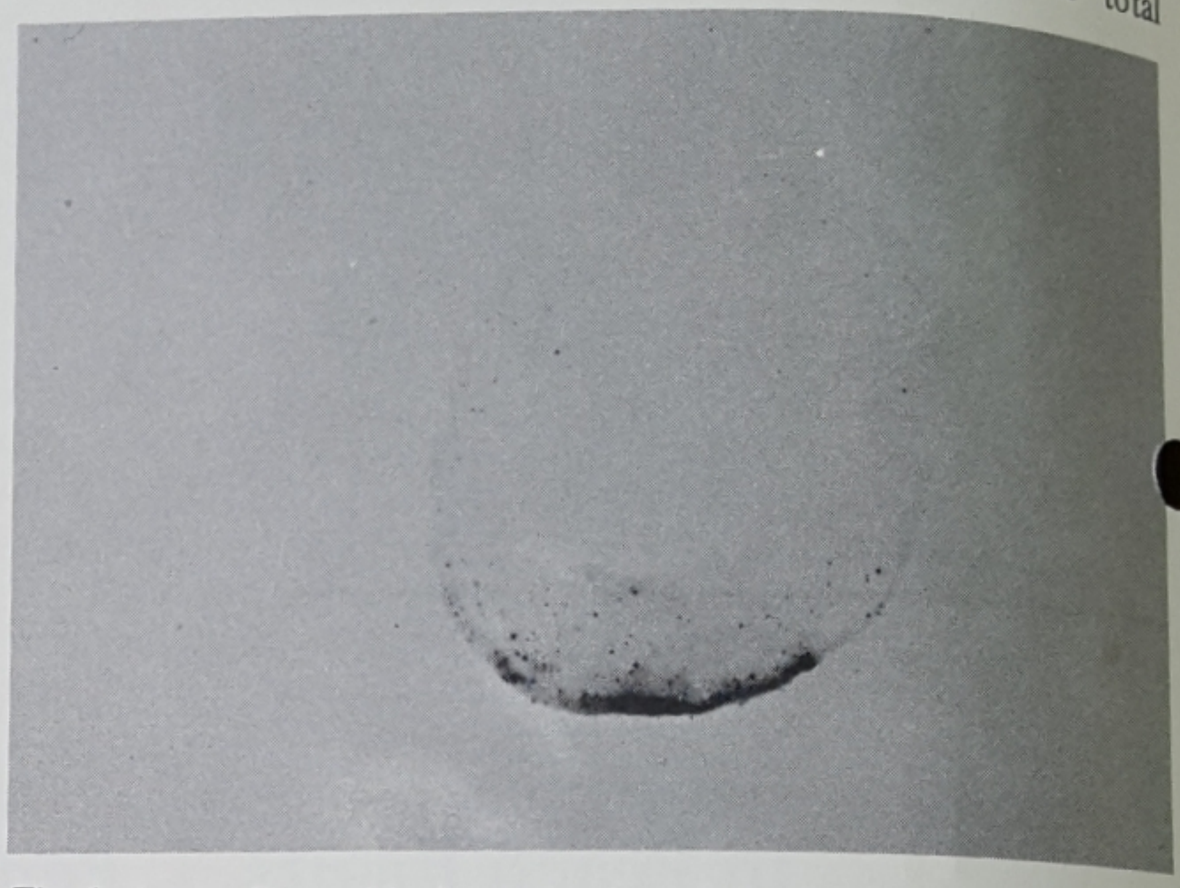

Fig. 1. Autoradiographic determination of distribution of radioactivity in prints of sections of 'Halford' fruits treated with 2 μCi of ^{14}C-CEPA on June 23, 1972, and harvested August 28. Film exposure time 21 days. Treated side of fruit at bottom of photograph.

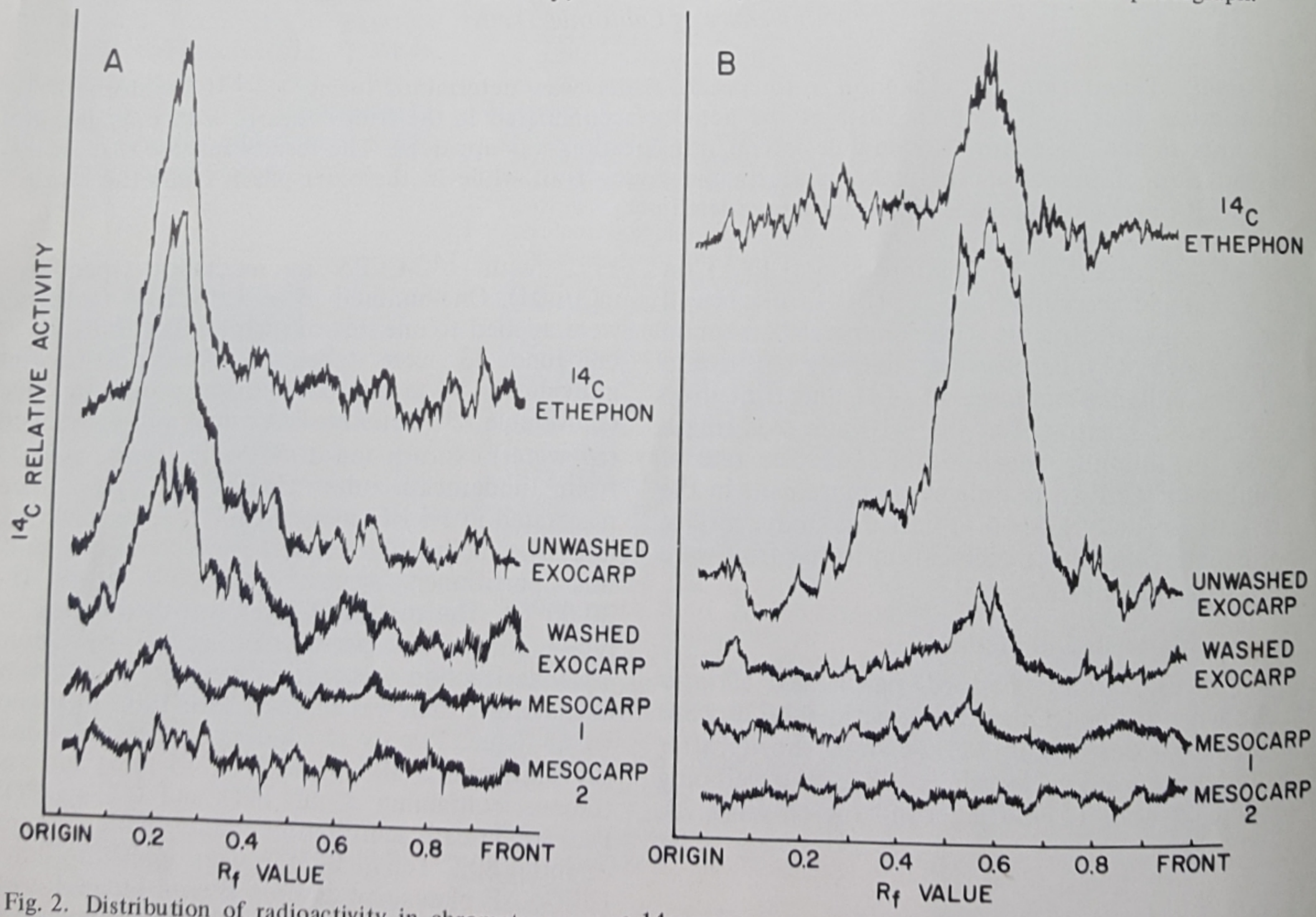

Fig. 2. Distribution of radioactivity in chromatograms of ^{14}C-CEPA standards and extracts of treated 'Halford' fruits. Fruits treated June 23, 1972, harvested August 28. Radioactive methanolic fraction chromatographed on Gelman ITLC and chromatograms assayed with TLC counter. A: developed in isopropanol:ammonia:water (8:1:1 v/v/v). B: developed in n-butanol:acetic acid:water (40:11:29 v/v/v).

Table 2. The relation between age and size of 'Halford' fruits and loss of radioactivity in ^{14}C-CEPA-treated fruits. (Fruits treated May 15, 1972.)

Harvest (days after treatment)	Radioactivity recovered (cpm/g fr wt) Exocarp[y]	Mesocarp[y,z]	Fruit surface ($mm^2 \times 10^3$)	Fruit volume ($mm^3 \times 10^3$)	Flesh volume ($mm^3 \times 10^3$)
21	126,710	4,700	16	195	403
45	32,360	3,618	18	230	196
70	26,720	910	31	524	490
95	22,810	392	62	1,437	1,403

[z]2 mm mesocarp layer below the exocarp.

[y]Mean separation by Duncan's multiple range test at the 5% level.

radioactivity was in the ^{14}C-CEPA region, with 2-5% at other locations on the chromatogram.

During growth and development of the fruits a marked decrease in radioactivity, on a fresh wt basis, occurred in the treated area (Table 2). Fruits harvested 45 days after treatment had only 25% as much radioactivity per g treated exocarp as did fruits harvested after 21 days, thereafter the reduction in radioactivity was considerably smaller (Table 2). The major reduction in radioactivity in the fruit mesocarp started after 45 days.

The reduction in radioactivity in the exocarp cannot be explained by dilution due to growth. The increase in exocarp area (fruit surface column of Table 2) was about 60% between 45 and 70 days after treatment, and 100% between 70 and 95 days. The reduction in radioactivity, however, did not exceed 17% during either of these periods. Furthermore, the major reduction of nearly 74% occurred during the early period between 21 and 45 days after treatment, when the increase in fruit surface was 12%. In the mesocarp, however, dilution of radioactivity with growth might have been a major factor in reduction of radioactivity, when calculated on a fresh wt basis. A fairly high correlation was found between the increase in mesocarp volume during the growth period and the reduction of radioactivity in the mesocarp (Table 2). Results of autoradiographs of thin fruit slices show a rather clear dilution of the radioactivity with the growth of the mesocarp and exocarp (Fig. 3).

^{14}C-CEPA is absorbed, translocated, and metabolized differently in different tissues, and usually absorption of the chemical is rather limited and slow (1, 6, 12, 15, 16). In our results, the relatively small amounts absorbed by peach fruits penetrated during the early stages of growth and/or were metabolized as shown by comparing radioactivity of the exocarp and mesocarp (Table 2). Although translocation of ^{14}C-CEPA has been reported from leaves of grape vines (15), walnuts (12), apples (6) and recently peach (1), in our study only a limited amount of material moved through the exocarp to the mesocarp. The reduction in radioactivity per unit fresh wt could have been due to the dilution effect of fruit growth. However, in the exocarp of young fruits, which contained most of the radioactivity, the reduction in radioactivity occurred irrespective of growth. In contrast to results with walnuts (12), tomato and squash (16), little metabolic loss of ^{14}C-CEPA from the mesocarp was detected, with most of the radioactivity remaining at the Rf of ^{14}C-CEPA. Metabolites of ^{14}C-CEPA have been reported in squash leaf (16), and peach fruit (1). Also, in this work where the ^{14}C-CEPA was applied to the fruit itself, there is evidence that not all the radioactivity could be recovered as ^{14}C-CEPA. However, the significance of these substances found in most cases in rather small amounts and their metabolic involvement is yet to be clarified.

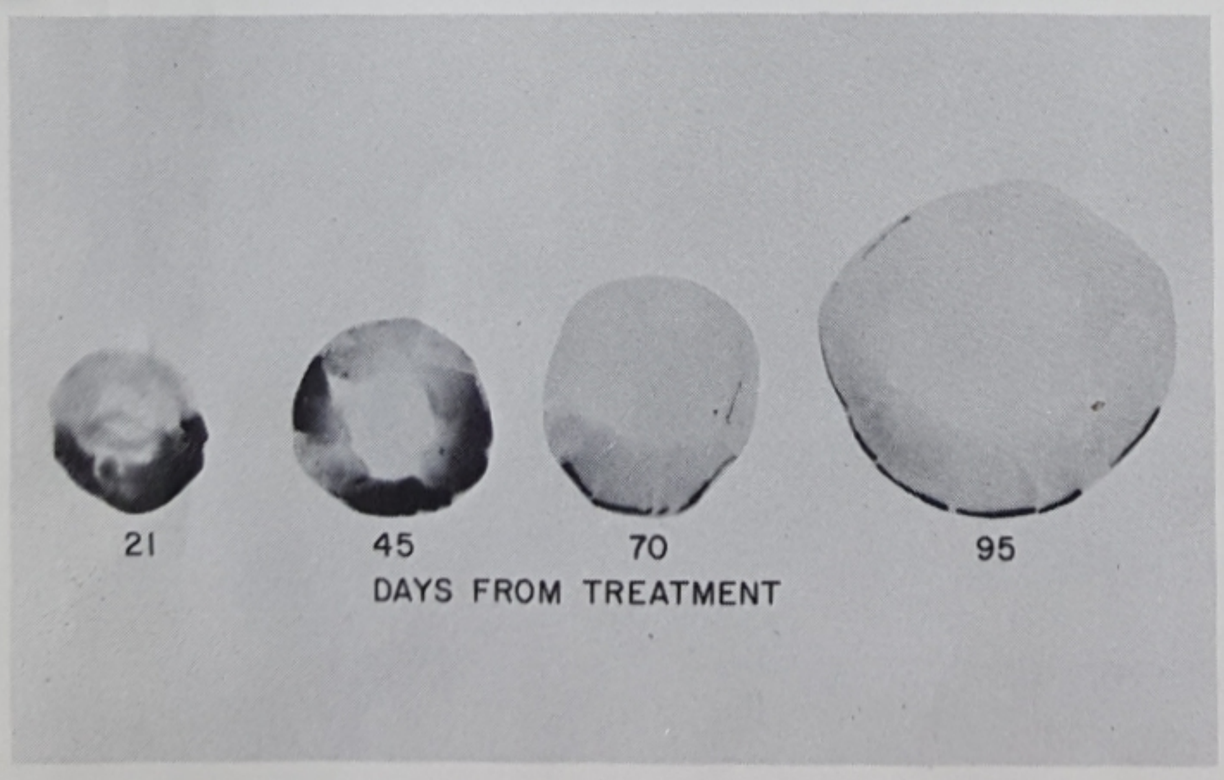

Fig. 3. Autoradiograms of prints made by sections of 'Halford' peach fruits treated with ^{14}C-CEPA. Fruits treated May 15, 1972, harvested 21, 45, 70 and 95 days later. Imprints on Whatmann 3 MM paper. Film exposure time 21 days. Treated side of fruit at bottom of photograph.

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