

**Table IV. Identification of C<sup>14</sup> in Plant Extracts as Dalapon-C<sup>14</sup> by Paper Chromatography**

Plant	Solvent System	Ex-tract <sup>a</sup>	R <sub>f</sub>	
			Dalapon reference	Pyruvic acid reference
Soybean	A	0.72 <sup>b</sup>	0.73	0.26
	B	0.69 <sup>b</sup>	0.70	0.56
	C	0.64 <sup>b</sup>	0.72	0.18
Corn	D	0.27 <sup>b</sup>	0.25	
	E	0.9 <sup>b</sup>	0.9	0.6
	E	0.9 <sup>c</sup>	0.9	0.6
	E	0.9 <sup>c</sup>	0.9	0.6

<sup>a</sup> Only one spot was found in each case.

<sup>b</sup> Water.

<sup>c</sup> Alcohol.

Solvent systems used for paper chromatography of extracts were:

- 100 ml. of *n*-butyl alcohol, 15 ml. of water, and 10 ml. of diethylamine.
- 250 ml. of methyl alcohol, and 1 ml. of 1*N* sodium ethylate.
- 100 ml. of *n*-butyl alcohol equilibrated with 100 ml. of 1.5*N* ammonium hydroxide.
- 50 ml. of chloroform, 50 ml. of *tert*-butyl alcohol, 5 ml. of ethyl alcohol, 1 ml. of diethylamine, and saturated with water.
- 100 ml. of 1-pentanol equilibrated with 100 ml. of 5*M* aqueous formic acid (4).

R<sub>f</sub> values of radioactive components on the chromatograms were computed from loci determined by radioautography or Geiger-Müller tube scanning.

### Results of Plant Experiments

Radioactivity entered soybean and corn plants whose roots were exposed to dalapon-C<sup>14</sup>. It was distributed throughout the plants as indicated in Tables I and II and Figure 5. There were higher concentrations in young tissues. The percentages of applied activity taken into the roots and into the tops for soybean were 0.6 and 2, respectively. For corn, under slightly different conditions, percentages were about 0.5 and 9.

Radioactivity entered soybean plants, when spotted on a leaf, and was distributed throughout the plants as shown in Table III. Here again the young tissues had the highest concentrations of activity. The leaf opposite the treated leaf and the roots had the least activity. About 2% of the applied activity moved into parts of the plants other than the treated leaves.

Examination by paper chromatography of extracts prepared from root-treated soybean plants showed the presence of radioactivity which moved with R<sub>f</sub> values corresponding to the R<sub>f</sub> of known dalapon-C<sup>14</sup>. No other radioactive spots were observed (Table IV).

From water and alcohol extracts of corn plants, radioactivity was recovered. This activity moved on chromatograms with R<sub>f</sub> values corresponding to that of dalapon (Table IV, Figure 6). No other compounds were found. Two plausible breakdown products, lactic and pyruvic acids, move in system E with R<sub>f</sub> values (0.62 and 0.65, respectively) well below the R<sub>f</sub> of dalapon (0.9).

### Literature Cited

- Bass, S. L. (to The Dow Chemical Co.), U. S. Patent 2,010,685 (Aug. 6, 1935).
- Bass, S. L., Burlew, W. L. (to The Dow Chemical Co.), *Ibid.*, 1,993,713 (March 5, 1935).
- Brust, H. F., Senkbeil, H. O. (to The Dow Chemical Co.), *Ibid.*, 2,809,992 (Oct. 15, 1957).
- Buch, M. L., Montgomery, R., Porter, W. L., *Anal. Chem.* 24, 489-91 (1952).
- Calvin, M., Heidelberger, C., Reid, J. C., Tolbert, B. M., Yankwich, P. F., "Isotopic Carbon," pp. 208-9, Wiley, New York, 1949.
- Claisen, L., Shadwell, J., *Ber.* 11, 1563-8 (1878).
- Crafts, A. S., Foy, C. L., *Down to Earth* 14 (4), 2-6 (1959).
- Denison, F. W., Jr., Phares, E. F., *Anal. Chem.* 24, 1628-9 (1952).
- Donaldson, K. O., Tulane, V. J., Marshall, L. M., *Ibid.*, 24, 185-7 (1952).
- Eidinoff, M. L., *Ibid.*, 22, 529-34 (1950).
- Foy, C. L., Ph.D. dissertation, University of California, Davis, Calif., 1958.
- Klimenko, E., *Ber.* 3, 465-8 (1870).
- Shive, J. W., Robbins, W. R., *New Jersey Agr. Expt. Sta. Bull. No. 636* (1948).
- Van Slyke, D. D., Folch, J., *J. Biol. Chem.* 136, 509-41 (1940).
- Van Slyke, D. D., Plazin, J., Weisinger, J. R., *Ibid.*, 191, 299-304 (1951).

Received for review July 13, 1959. Accepted December 9, 1959.

## FUMIGANT RESIDUES

### Retention of Acrylonitrile and Carbon Tetrachloride by Shelled Walnuts Fumigated with Acrylon

IN EXPLORATORY TESTS with four fumigants to control infestations of the Indian-meal moth, *Plodia interpunctella* (Hbn.), in imported shelled walnuts, Acrylon provided 100% control with no undesirable effects on flavor, odor, or appearance (7). A feature of the experiment was the successful use of polyethylene bags as fumigation chambers as described below. Acrylon is also known as Acritet (Stauffer Chemical Co., New York) and consists of acrylonitrile and

carbon tetrachloride, 34 to 66% by volume, or 20.4 to 79.6% by weight.

Acrylon applied at the rate of 3 ml. per 55 pounds (4.1 grams per 25 kg.) of nut meats for an exposure period of 16 hours, was consistently successful in controlling infestations during a 3-year period in which some 500,000 pounds of shelled walnuts were treated (13). However, information was needed on the residues of acrylonitrile and of carbon tetrachloride that remained after aeration and

BEN BERCK

Canada Department of Agriculture,  
Research Station, Winnipeg, Man.,  
Canada

packaging of the treated nuts. This report deals with concentration-time relationships for the Acrylon components over a period of 38 days.

#### Methods

**Fumigation under Atmospheric Pressure.** Double-walled polyethylene bags, large enough to accommodate the contents of a 55-pound box of shelled walnuts, served as small and inexpensive

Imported shelled walnuts, in 55-pound batches, were successfully fumigated in polyethylene bags to control insect infestation by injecting each bag with 3 ml. of Acrylon. Desorption rates of acrylonitrile and carbon tetrachloride during a 38-day storage period showed that their residual amounts were affected by fumigant dosage, duration of exposure, and aeration by fan. Fumigation under reduced pressure, followed by storage for 30 days, resulted in acrylonitrile and carbon tetrachloride residues which were lower than those obtained by fumigation at atmospheric pressure. Acrylonitrile was determined polarographically and carbon tetrachloride spectrophotometrically with satisfactory specificity and sensitivity.

fumigation chambers. A wad of cotton batting backed by a double layer of aluminum foil was placed with cotton side out in the center of one of the sides of the bag. The bag opening was folded and tied tightly with flexible metal ties, and each bag was placed horizontally on a platform in the vicinity of an exhaust fan.

Acrylon was dispensed with a 5-ml. glass syringe fitted with a mechanical stop at a precalibrated 3-ml. mark, after Chaney (3), and with a No. 18 stainless steel hypodermic needle to pierce the bag at the target center. After the liquid was discharged, the needle was withdrawn, the hole sealed with Scotch cellulose tape, and the process repeated for each bag.

After exposure to the fumigant for 16 hours at 70° to 75° F., the walnuts were spread out on a slat platform covered with cheesecloth and aerated for 4 hours in front of a floor-type fan with 24-inch diameter blades and adjustable oscillation within a maximum arc of 90°. The aeration was followed by air-cleaning with a fanning mill to remove loose nut tissues, insect fragments, etc. Finally, the walnuts were mechanically packed in cellophane bags in half-pound lots and stored on a laboratory shelf at room temperature. Samples from different bags were analyzed at predetermined intervals. The standard or normal conditions that were tentatively adopted were 3 ml. of Acrylon per 55 pounds of shelled walnuts, 16 hours' exposure in the sealed polyethylene fumigation chambers, and 4 hours' aeration by fan. Variations of these conditions were studied to find the effect of doubling the normal dosage rate, tripling the normal exposure period, and eliminating the aeration by fan.

**Vacuum Fumigation.** In vacuum fumigation, wherein the air pressure is reduced before the fumigant is released, diffusion and penetration proceed rapidly; therefore, the period of exposure may be shortened considerably. Although methyl bromide appears to be the most widely used fumigant for vacuum fumigation, Monro suggested (11) determining acrylonitrile and carbon tetrachloride residues of walnuts fumigated with Acrylon under reduced pressure.

A 25-quart cast aluminum pressure canner, *A*, was converted for vacuum fumigation, following suggestions by Lynn (9) and Monro (11). The pressure fittings were removed from the cover and were replaced with cemented glass and metal fittings as shown in Figure 1. A cheesecloth bag, *C*, containing 10 pounds of shelled walnuts and six cages, each of which contained 50 adults of confused flour beetles, *Tribolium confusum* Duv., 2 weeks old, was placed on the wire rack, *B*. To promote uniformity of the gas-air mixture an improvised fan made of a thin brass sheet, *D*, soldered to a flexible metal strip was attached to a swivel that was suspended on the underside of the cover, *E*. The fan was operated by an Alnico magnet moved manually on the dome of the chamber.

With the cover in position a weighed amount of Acrylon liquid corresponding to 78 mg. per liter of space in the fumigation chamber was contained in the borosilicate glass vessel, *F*. After the vessel was evacuated, *J*, to 75 mm. of mercury, Acrylon was introduced as a gas through butt joint *H*, and the copper gas-inlet tube, *I*. The evaporation of Acrylon liquid was accelerated by immersing the glass vessel in lukewarm water. When evaporation was visibly complete, the air was admitted, *G*, until a vacuum of 100 mm. of mercury was

reached (*K*, vacuum gage). The Alnico magnet was moved to and fro for 5 minutes to cause movement of the improvised fan to assist admixture of gas and air. After 3 hours of fumigation at 70° F., air was admitted, the cover was removed, and the nut meats were sampled for analysis and the effect of aeration.

#### Method for Carbon Tetrachloride.

The residual amounts of carbon tetrachloride retained by the fumigated walnuts were determined spectrophotometrically by the Ramsey method (12), employing the Fujiwara reaction.

Exploratory tests with carbon tetrachloride and acrylonitrile standards added to untreated walnuts showed that the efficiency of recovery of carbon tetrachloride was 85 to 90%, and that acrylonitrile did not interfere, even if present at 50 times the carbon tetrachloride levels. Amounts of carbon tetrachloride as low as 0.1 mg. were readily measured by the Fujiwara reaction, with a variation between triplicates not exceeding 0.7% transmittance.

A 100-gram sample of shelled walnuts was lightly rolled with a glass bottle to reduce the particle size and was placed in a two-necked 1-liter round-bottomed flask to which 265 ml. of distilled water, 30 ml. of 1*N* sulfuric acid, and 5 ml. of 20% phosphotungstic acid were added. From this point Ramsey's procedure (12)

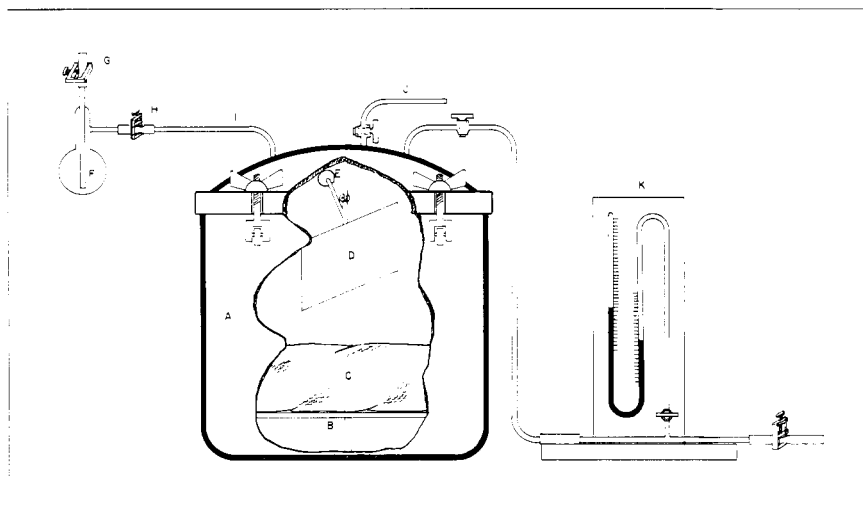


Figure 1. Vacuum fumigation assembly

was followed, except that a more rapid rate of air flow of approximately 100 ml. per minute was preferred. Also, a constant air suction source regulated with a needle valve and rotameter provided steadier air flow than was attained with a Mariotte bottle. A Beckman B spectrophotometer with 10-mm. matched cells was used to measure the color at a wave length of 530 m $\mu$ . As the slope of the standard curve varied somewhat on different days, for each group of determinations a fresh standard curve was used, along with carbon tetrachloride and carbon tetrachloride-acrylonitrile check tests, to cancel out small differences between days. Slight turbidity of the pyridine-alkali reagent sometimes occurred, and this was corrected by filtering through a dry, folded filter paper. The analytical results were corrected for the value of blank (untreated) samples and for recovery of carbon tetrachloride from added standards.

**Acrylonitrile.** The polarographic method of Fuller and Norris (5) was adapted for the determination of acrylonitrile retained by the Acrylon-fumigated walnuts. The method depends on the fact that acrylonitrile forms an azeotropic mixture with water and can thus be concentrated and codistilled out of aqueous suspensions of foodstuffs, etc. Acrylonitrile reduces at about -1.90 volts in a polarographic cell employing a dropping mercury electrode and a mercury pool anode, with 0.1M tetramethylammonium hydroxide as the supporting electrolyte.

The Gawron polarographic cell (6) used was simple and convenient for routine work and gave reproducible results. Although acrylonitrile may be measured in the presence of dissolved oxygen, the recorded waves were more reproducible when the cell solution was deaerated with nitrogen (it was not necessary to deaerate the anode solution). All measurements were made with a Radiometer Model PO<sub>3</sub> recording polarograph (Radiometer Co., Copenhagen, Denmark).

Standard solutions of acrylonitrile in

water were prepared from a purified and freshly distilled sample of acrylonitrile provided by the Research Division, American Cyanamid Co., Stamford, Conn. Amounts of acrylonitrile as low as 0.025 mg. were readily measured. The efficiency of recovery of acrylonitrile standards added to aqueous suspensions of walnuts was 95 to 100%, with no interference from carbon tetrachloride. Calibration data showed a linear relationship between concentrations of acrylonitrile in the original solution and the diffusion current of a given fraction or aliquot.

For separating acrylonitrile from the treated walnuts, distillation from an acidified water suspension was employed. Thus, a 100-gram sample was placed in a 24/40 1-liter round-bottomed flask, to be heated with an electrical heating mantle. A solution of 280 ml. of distilled water, 15 ml. of 1N sulfuric acid, and 5 ml. of 20% phosphotungstic acid was added. A 250-mm. Vigreux fractionating column, a 75°-angle condenser head, a 300-mm. Liebig condenser with adapter, and a graduated 25-ml. receiver immersed in an ice bath were attached. The distillation was regulated to about 1 ml. per minute with a voltage regulator, until about 23 ml. of distillate were collected. This was diluted to 25 ml. in a volumetric flask, from which a suitable aliquot (between 1 and 5 ml. generally provided an adequate sample) was transferred to a 10-ml. volumetric flask. One milliliter of 1M tetramethylammonium hydroxide, polarographic grade (Southwestern Analytical Chemicals, Austin, Tex.) was added to the aliquot, and then diluted to the mark with water, and mixed and transferred to the Gawron cell (9) containing 0.1M tetramethylammonium hydroxide in the anode compartment. The cell solution was deaerated with nitrogen pre-saturated by passing through a separate portion of a blank solution. Current-voltage curves from -1.4 to -2.2 volts were recorded in the usual manner, measuring the diffusion current as the differ-

ence between the base line (residual current) and the plateau of the acrylonitrile wave (at about -2.0 volts).

## Results

The concentration-time relationships of carbon tetrachloride and acrylonitrile that resulted from the various treatments applied to shelled walnuts are shown in Tables I and II.

The mortality of test insects in all cases was complete. The mortality resulting from vacuum fumigation with the relatively short exposure period of 3 hours was interesting in that examination of the test cages 1/2 hour after opening the chamber showed that the cages located at the perimeter of the load had a larger percentage of affected insects. However, all the test insects were dead 7 hours after removal from the toxic atmosphere.

## Discussion

Table I, dealing with carbon tetrachloride and acrylonitrile levels of walnuts fumigated in polyethylene bags under atmospheric pressure, shows that dosage, period of exposure, and aeration influence the amounts retained by the packaged walnuts. Thus, when the dosage was doubled and the exposure period was increased 2.7 times without supplementary aeration by fan, the carbon tetrachloride residue levels over the 38-day period were appreciably higher, although not in direct proportion to the increase in dosage and exposure period. Aeration by fan, however, lowered the carbon tetrachloride residues in every instance. Although for this experiment both the fan and the aeration period were arbitrarily chosen, nevertheless it is suggested that lower residues might be obtained in commercial practice by adapting "fluidized solid bed" columns (10) for forced aeration. In any event, the normal or standard conditions that were chosen for commercial application (7)

**Table I. Amount of Acrylon Components Found on Atmospheric-Fumigated Walnuts**

(Interaction of 2 dosages  $\times$  2 exposure periods  $\times$  2 aeration periods)

Nature of Treatment			Days after Packaging of Walnuts											
Acrylon added, <sup>a</sup> ml.	Exposure, hr.	Fan, hr.	Carbon Tetrachloride, P.P.M.						Acrylonitrile, P.P.M.					
			2	9	18	24	30	38	2	9	18	24	30	38
3	48	4	28.0	25.5	16.0	13.0	9.6	4.8	7.5	5.0	0.0	0.0	1.0	2.5
3	48	0	40.5	32.2	24.5	20.9	23.1	24.0	10.0	5.0	0.8	1.0	1.0	2.0
6	48	4	33.0	30.0	10.0	17.5	9.6	14.4	10.0	6.0	2.5	2.5	3.5	3.7
6	48	0	50.4	33.0	35.2	...	33.7	34.5	17.5	11.0	6.8	10.0	10.5	8.5
3	18	4	15.7	6.7	6.0	4.7	2.9	5.7	5.0	1.0	0.0	1.0	0.0	0.0
3	18	0	18.0	18.5	17.2	15.1	13.5	13.0	6.0	1.0	0.0	2.5	1.0	0.5
6	18	4	18.0	24.0	18.0	13.0	11.5	13.3	11.0	12.5	2.4	4.0	3.5	1.0
6	18	0	28.2	39.5	35.0	31.8	24.0	21.6	13.5	12.5	4.1	5.0	7.5	5.0

<sup>a</sup> Normal dosage = 3 ml. Acrylon per 55 pounds of walnuts, or 164 mg. per kg. of walnuts.

**Table II. Amount of Acrylon<sup>a</sup> Components Found on Vacuum-Fumigated Walnuts<sup>b</sup>**

Nature of Treatment	Days after Packaging of Walnuts													
	<sup>3</sup> / <sub>4</sub>	1	4	8	14	21	30	<sup>3</sup> / <sub>4</sub>	1	4	8	14	21	30
	Carbon Tetrachloride, P.P.M.						Acrylonitrile, P.P.M.							
Treatment A <sup>c</sup>	17.5	16.0	13.5	9.3	10.5	7.3	5.3	12.5	16.6	16.6	14.9	6.8	4.1	1.3
Treatment B <sup>d</sup>	16.5	12.0	5.0	1.9	2.4	0.5	0.5	16.0	12.5	10.0	6.0	2.5	0.8	0.0

<sup>a</sup> Total weight of Acrylon applied = 1.882 grams; applied dosage = 1882/4.55 = 413 mg./kg.

<sup>b</sup> Total amount of nuts in 24.1-liter vacuum-fumigation chamber = 10 lb. or 4.55 kg.

<sup>c</sup> Vacuum-fumigated for 3 hours at 110 mm. of mercury at a relative concentration per liter of empty space = 1882/24.1 = 78 mg./liter.

<sup>d</sup> Same as Treatment A, but aerated in laboratory fume hood for 16 hours.

(3 ml. of Acrylon per 55 pounds of walnuts, 16 hours' exposure, and 4 hours' aeration by fan) gave the lowest carbon tetrachloride residues.

Table I also indicates that the residue levels of acrylonitrile are appreciably lower than those of carbon tetrachloride, and that aeration assists the lowering. With due regard to the greater relative toxicity of acrylonitrile, and also to the 1 to 4 ratio of acrylonitrile to carbon tetrachloride as initially applied, the comparative rate data shown herein confirm the author's findings with wheat (2) that the ratio of the components of a fumigant mixture may change considerably after application because of differential sorption.

Table II, pertaining to vacuum fumigation, shows generally lower carbon tetrachloride residues than those of atmospheric-fumigated walnuts, despite the greater amount of fumigant that was applied. Evidently the shortened exposure period was a major factor herein. Supplementary aeration, as in treatment B, aided the lowering of residues of both carbon tetrachloride and acrylonitrile, and confirms the trend shown in Table I. The vacuum of 110 mm. of mercury that was used in this experiment approximates the 25-inch vacuum used commercially.

As indicated previously, methyl bromide is widely used for fumigation of nut meats and other foodstuffs. Because of differences in conditions and methods of fumigation, it is not possible to make valid comparisons of the concentration-time relationships between the bromide residues of walnuts reported by others

(4, 7, 8) and those of acrylonitrile and carbon tetrachloride reported herein. It would appear, nevertheless, that the desorption patterns for methyl bromide residues of vacuum-fumigated walnuts show a sharp drop within 24 hours from relatively high values and become almost constant thereafter to levels of 30 to 40 p.p.m. (7).

Polyethylene bags proved to be simple, inexpensive, and convenient as fumigation chambers for fumigation of 25-kg. batches of walnuts that had been stored in their original boxes in commercial cold storage at 34° F., and which were treated as required at the packaging warehouse (13). The method of application by syringe is considered to be simple, inexpensive, and safe, if adequate precautions are taken in the application. This method would not be suitable for extremely volatile fumigants such as methyl bromide.

Forced aeration applied within several hours after the exposure period should be investigated in greater detail as a means of lowering volatile fumigant residues.

#### Acknowledgment

The author acknowledges the assistance provided by J. M. Sinclair, Ltd., Winnipeg, in the developmental studies with polyethylene bags, the suggestions of H. A. U. Monro, Canada Agriculture Research Branch, London, Ontario, regarding vacuum fumigation, and the outline of an analytical method for acrylonitrile and research samples pro-

vided by M. V. Norris, American Cyanamid Co., Stamford, Conn.

#### Literature Cited

- (1) Berck, B., Can. Agr. Research Branch, Winnipeg, Man., unpublished data.
- (2) Berck, B., *Proc. Xth Intern. Congr. Entomology* **4**, 99 (1956).
- (3) Chaney, A. L., *Ind. Eng. Chem., Anal. Ed.* **10**, 326 (1938).
- (4) Dudley, H. C., Miller, J. W., Neal, P. A., Sayers, R. R., *Pub. Health Repts. (U.S.)* **55**, 2251 (1950).
- (5) Fuller, L. T., Norris, M. V., American Cyanamid Co., Research Div., Stamford, Conn., private communication.
- (6) Gawron, O., *Anal. Chem.* **22**, 614 (1950).
- (7) Gerhardt, P. D., Lindgren, D. L., Sinclair, W. B., *J. Econ. Entomol.* **44**, 384 (1951).
- (8) Laug, E. P., *Ind. Eng. Chem.* **33**, 803 (1941).
- (9) Lvn, G. E., *Down to Earth*, **5**, 3, 5 (1949).
- (10) Mathur, K. B., Gishler, P. E., *J. Appl. Chem. (London)* **5**, 624 (1955).
- (11) Monro, H. A. U., Pesticide Research Inst., Can. Dept. Agr., London, Ontario, private communication.
- (12) Ramsey, L. L., *J. Assoc. Offic. Agr. Chemists* **40**, 175 (1957).
- (13) Sinclair, J. M., J. M. Sinclair, Ltd., Winnipeg, Man., personal communication.

Received for review July 24, 1959. Accepted October 23, 1959. Division of Agricultural and Food Chemistry, 136th Meeting, ACS, Atlantic City, N. J., September 1959. Contribution No. 30, Can. Dept. of Agriculture, Research Station, Winnipeg, Man., Canada.