Fate of Pentachlorophenol in Cotton

Charles S. Miller and Mohamed M. Aboul-Ela[1]

Pentachlorophenol (PCP) or its metabolites accumulated in quantities up to 2 p.p.m. in cottonseed kernels of bolls which were closed when sprayed, but was not detected in kernels from open bolls on the same cotton plants. Injecton of closed bolls with formulated PCP in diesel oil produced abortion of seed, but seed weight was not correlated with PCP content. When applied as single droplets to leaf blades, PCP accumulated in the glandular hairs along the veinlets, thus reducing the quantities translocated. Oat seedlings grown in pots with gram quantities of burrs which contained 11.9 p.p.m. of PCP equivalents did not absorb above 0.5 microgram.

Pentachlorophenol (PCP) was first synthesized in 1841 (Carswell and Nason, 1938), but was not manufactured in quantity until 1936. Its uses included weed control in sugarcane and right-of-ways; slime and algae control; preservative for wood, textiles, and leather goods. Previous studies with PCP include its decomposition in soil (Young and Carroll, 1951), influence on plant cells (Currier, 1949), and mobility in wood as influenced by different solvents (Hatfield and Van Allen, 1958). In 1950, the use of PCP as a desiccant for cotton became established (Jaworski, 1955) to assist in the preparation of plants for mechanical stripper harvest. Jaworski (1955) concluded that there was little if any translocation of PCP in cotton plants and reported quantities of less than 0.01 p.p.m. in cotton seed oil of field-grown plants sprayed with PCP-1-C^{14}.

The experiments reported here were done to increase the understanding of the absorption, translocation, and residue aspects of PCP in cotton.

MATERIALS AND METHODS

Pentachlorophenol-1-C^{14} (specific activity of 1 mCi./mM.) was formulated to contain 40.5% PCP, 20.0% diacetone alcohol, and 39.5% Shell 10 medium aromatic oil on a weight/weight basis. This stock solution was diluted 30 to 1 with No. 2 diesel oil for spraying and injection of bolls. The specific activity of the spray solutions was 55 μCi. per ml.

Mature Plant Residue Studies. In two separate experiments, PCP-1-C^{14} was sprayed on greenhouse-grown, mature, Deltapine variety cotton plants. In each experiment, three plants which were grown in 1-gallon plastic pots filled with peat moss and perlite were used. In the first experiment, the plants averaged 25 bolls per plant and in the second, 10 bolls per plant. Approximately 30% of the bolls were open at time of application of the sprays in both experiments.

The harvest times were 9, 19, 26, 34, and 40 days from spraying for the first experiment and 12, 53, and 84 days in the second. The 84-day harvest represented bolls which were developed from squares after treatment.

The seed cotton of the bolls was harvested individually, ginned, and the seed hulled with a scalpel. Representative samples of lint and burrs, hulls and kernels from each boll were radio assayed.

Injected Bolls. Twenty bolls, 2.5 to 3.0 cm. in diameter, on five plants were individually treated with an aliquot of the spray solution from the second experiment. The same proportions and specific activities were maintained in the preparation of the spray solution for the second experiment. Half of the bolls were injected with 0.04 ml., and the other half were painted with 0.08 ml. each. After harvest 8 to 23 days later, the bolls from each treatment were combined and representative samples of kernels from the seed were radioassayed. An aliquot of the spray solution from the second experiment was used for injection of 5 or 6 closed bolls on each of four mature Deltapine variety cotton plants. A hypodermic syringe fitted with a needle and micrometer was used to inject 0.02 ml. into each of two carpels per boll. The injected bolls were harvested 36 and 52 days after treatment. The day of opening was recorded for each boll. The seed cotton from each lock was separately ginned and radioassayed.

About 50 days later, a few newly developed bolls on the same plants were injected with 0.04 ml. of the PCP-1-C^{14} in one carpel only for subsequent harvest and analysis.

Translocational Studies. Seedlings. Several 12-day-old Deltapine cotton seedlings were treated with two preparations of PCP-1-C^{14}. The first preparation was made by diluting the second spray solution 1 to 10 with diesel oil. The other treatment solution was prepared by dissolving PCP-1-C^{14} in absolute ethyl alcohol. The concentration of PCP and the specific activities of the two preparations were essentially equal. Individual drops of the preparations were applied with capillary tubes either to the point of veinal anastimosis of the first true leaf or at the juncture of the cotyledon and its petiole.

Treated seedlings were harvested at intervals from one hour to eight days and exposed to X-ray film at −20 C.

Seedlings Grown from Seed of Sprayed Plants. Twenty-five seeds which were taken from 25 different bolls from the three plants sprayed in the first experiment were saved for germination. Six of the seedlings which were grown for 13 days after germination were harvested and autoradiographed.

Regrowth of Sprayed Plants. Four representative samples of regrowth leaves on sprayed plants in the second experiment were harvested 84 days from treatment. dried under an infrared lamp, and radoassayed.

Residual Effect of PCP in Soil. Twelve burrs from the second spraying, which contained an average of 11.9 p.p.m. PCP equivalents and a specific activity of 0.13 μCi. per gram, were ground in a Wiley mill to pass a 20-mesh screen. Approximately 1 gram of the ground burrs was mixed with the top $\frac{1}{2}$ inch of soil in 4 pots, then covered with $\frac{1}{4}$ inch of soil. Oats of an unknown variety were planted, covered with $\frac{1}{2}$ inch of the same soil, and watered with distilled water as

Department of Plant Sciences, Texas A&M University, College Station, Texas 77843

[1] Present address, Department of Biology, Texas Woman's University, Denton, Texas

Table I. Equivalent PCP Content of Boll Components from PCP-1-C^{14} Sprayed, Greenhouse-Grown Deltapine Variety Cotton Plants

Experiment	Days from Spraying to Harvest	Boll Condition at Spraying	Kernels		Hulls		Lint		Burrs	
			No.	P.P.M.	No.	P.P.M.	No.	P.P.M.	No.	P.P.M.
I	9	Open	23	<0.40[a]	23	1.90 ± 0.34	23	24.04 ± 7.35	23	5.88 ± 0.75
	9	Closed	1	<0.40	1	2.80	1	11.85	...	...
	19	Closed	5	1.28 ± 0.12	5	0.55 ± 0.09	5	1.00 ± 0.27	3	12.80 ± 3.14
	26	Closed	6	2.17 ± 0.31	6	0.81 ± 0.16	6	1.16 ± 0.36	4	9.86 ± 3.75
	34	Closed	5	2.02 ± 0.14	5	1.07 ± 0.08	5	1.56 ± 0.20	5	10.62 ± 4.45
	40	Closed	6	1.79 ± 0.12	6	1.54 ± 0.21	6	1.83 ± 0.23	5	12.39 ± 3.24
	44	Closed	2	1.17 ± 0.16	2	0.80 ± 0.08	2	1.26 ± 0.10	1	19.40
II	12	Open	6	<0.40	6	1.26 ± 0.76	6	7.12 ± 3.43	6	7.58 ± 0.84
	12	Closed	6	<0.40	6	<0.40	6	0.69 ± 0.26	5	28.22 ± 14.60
	53	Closed	6	1.38 ± 0.36	6	0.73 ± 0.19	6	1.39 ± 0.60	6	3.05 ± 0.11
	84	Buds[b]	1	0.55	3	<0.40	3	<0.40	3	1.04 ± 0.42

[a] Lower limit of sensitivity expressed as PCP equivalents.
[b] Floral parts developed on subsequent new growth.

Table II. Equivalent Pentachlorophenol Content in Kernels from PCP-1-C^{14} Injected Bolls of Greenhouse-Grown Deltapine 15 Cotton Plants

Days from Injection to Opening	Injected Locks		Non-injected Locks	
	No.	Average p.p.m.	No.	Average p.p.m.
2–12	11	31.25 ± 14.43 $r = -0.327$ wt. mg. 134 ± 29	19	1.63 ± 0.67 $r = -0.279$ wt. mg. 204 ± 22
26–49	14	11.12 ± 9.56 $r = -0.398$ wt. mg. 105 ± 20	13	0.98 ± 0.42 $r = 0.139$ wt. mg. 204 ± 27

necessary. Three soil types, Norwood silty clay loam, Houston black clay, and builders sand, were used. An untreated control pot of each soil was included in the split plot design, four replication tests.

The oat seedlings were clipped above the soil level to avoid contamination and the replicates combined, dried, ground, and radioassayed at 10, 17, 25, 31, and 59 days from planting.

After the fifth harvest, each type of soil was washed with 500 ml. of distilled water and the leachate was reduced to 50 ml. and radioassayed.

Determination of Pentachlorophenol. The burrs, lint, hulls, and kernels were air-dried at room temperature. The burrs and hulls were ground on a laboratory mill, but the kernels were broken up with mortar and pestle. The ground samples were pressed into wafers and counted. The amount of radioactivity of unknown samples was compared with the amount of radioactivity from a comparable series of wafers made with known quantities of PCP-1-C^{14} (Corbett and Miller, 1963). The lower limit of sensitivity was 0.4 p.p.m. for the kernels and burrs, and 0.25 p.p.m. for the hulls and lint.

RESULTS

Mature Plant Residue Studies. The equivalent amounts of PCP in various parts of bolls from sprayed plants are presented in Table I. Most of the flowers and a few young bolls abscized before harvest in both experiments.

Injected Bolls. A summary of the results obtained from injected bolls is shown in Table II. As would be expected, the kernels within the injected carpels contained more of the PCP than kernels of seed from adjacent carpels. The seed

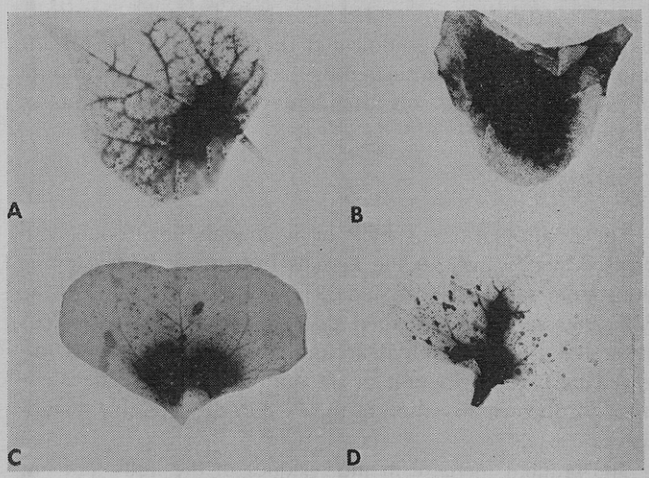

Figure 1. Autoradiographs of cotton leaves and cotyledons treated with PCP-C^{14}

A. First true leaf 3 days after treatment with formulated PCP in diesel oil
B. First true leaf 8 days after treatment with PCP in ethanol
C. Cotyledonary leaf 4 days after treatment with PCP in ethanol
D. Cotyledonary leaf 4 days after treatment with formulated PCP in diesel oil

of the injected carpels of the young bolls visually appeared poorly developed and in many instances the carpels did not dehisce. The poor development or abortion of the seed was not necessarily associated with higher carbon-14 contents, since none of the correlation coefficients between p.p.m. of PCP equivalents and milligrams weight of the kernels were significant (Table II).

Translocational Studies. Autoradiographs of young cotton seedlings treated with one droplet of PCP-1-C^{14} in diesel on their first true leaves showed some absorption and translocation through the veins of the treated first true leaves within 1 hour after treatment. After 8 hours, the radioactivity was distributed through all of the veins of the treated leaves, but there was no apparent movement out of the treated leaves even after 8 days (Figure 1).

Radioactivity was well distributed through the veins of the cotyledonary petioles wthin 4 hours after application. At the same time, there was evidence of movement of the labeled material into the cotyledonary petiole, stem, and apex of the seedling. By one day after application, all parts of the seed-

ling except the other cotyledon and roots contained observable carbon-14 activity.

PCP-1-C^{14} in ethanol was distributed similarly when applied to the primary leaves, but there was no movement from the treated cotyledonary blades within 8 days (Figure 1).

With both formulations, there was a tendency for the radioactivity to concentrate in the glandular hairs.

Seedlings Grown from Seed of Sprayed Plants. Germination of the seed harvested from sprayed cotton plants was 92% and only one of the 23 seedlings which emerged appeared weak. Autoradiographs of the subsequent seedlings indicated no apparent darkening, even though other seed from the same bolls contained the equivalent of 1.13 to 3.27 p.p.m. of PCP in the kernels.

Regrowth of Sprayed Plants. The average content of PCP equivalents in regrowth leaves from sprayed cotton plants was 1.03 p.p.m. in four samples, expressed on a dry weight basis.

Residual Effect of PCP in Soil. None of the oat seedlings grown on the three types of soil to which a sample of PCP-1-C^{14} sprayed burrs was added absorbed detectable quantities of radioactivity. This indicated that less than 4% of the applied dose was accumulated by the seedlings. The amount of carbon-14 in the soil leachates was below the limits of detection.

DISCUSSION

The accumulation of PCP or its metabolic products increased significantly in the kernels from seed of developing bolls from samples harvested 19 days after treatment to the 26th day samples and then declined after 40 days. This suggested either an initial accumulation in the kernels followed by a redistribution or an optimum stage of development for the accumulation of the PCP or any possible metabolites.

The gradual increase in the residues in the hulls and lint of closed bolls with decrease in the age of the bolls at time of spraying and the sharp decrease in younger (40th day harvest) ones is partial evidence of redistribution of carbon-14. However, these results may have been due to less spray deposit on the smaller bolls, which in their younger stages were enclosed by the bracts.

The consistently lower content of PCP or possible metabolic products in the hulls of closed bolls as compared to the kernels and lint cannot be fully explained, but may be an indication of translocation from the hulls to the developing lint fibers which originated in the hulls.

The variability of residues in the lint and burrs of open bolls is undoubtedly a reflection of the amount which they received during spraying.

Although many of the seeds of the injected bolls aborted, no correlation between the residue level and weight of the kernels was established. In the injected carpels, some of the poorly developed embryos contained less than 1 p.p.m. of the residues, while some of the better developed ones contained over 20 p.p.m. The same situation existed with the kernels of the seed of non-injected carpels, and thus the poor embryonic development was apparently not directly dependent on the residual content of the PCP in the kernels themselves. The arrested development of the kernels, then, was probably due more to injury of the funiculus than injury of the embryo.

The localization and lack of movement of PCP-1-C^{14} in diesel oil, when applied as a drop to primary leaves of young cotton seedlings, was an indirect indication of lack of metabolism. Concentration of the carbon-14 from PCP in the glandular hairs located along the supporting veins was probably due to diffusion and is undoubtedly a clue to the function of these structures.

The distribution of carbon-14 in mature plants following spray application of PCP-1-C^{14} indicated that there may be a slight amount of metabolism of PCP which proceeds at a slow rate or a small amount of redistribution from treated leaves. Since the redistributed carbon-14 was principally in the veins of the regrowth, it was assumed that this was not the result of volatilization and accumulation. These conclusions are evidenced by the determination of the presence of the equivalent of 1 p.p.m. of PCP in the new vegetative plant parts which sprouted several days after treatment.

The results of these experiments are similar to those conducted by Jaworski (1955), in that no detectable amounts of pentachlorophenol could be found in seed kernels of open bolls on plants which were sprayed. However, in contrast to his conclusions, it was observed that there could be some translocation of PCP or possible metabolic products within the plants and that PCP residues definitely existed in seed from bolls which were closed at time of treatment.

ACKNOWLEDGMENT

The authors express their appreciation for the technical assistance of Susan Clark and N. J. Pritchard.

LITERATURE CITED

Carswell, T. S., Nason, H. K., *Ind. Eng. Chem.* **30,** 622–6 (1938).
Corbett, J. R., Miller, C. S., *Nature* **199,** 1106–7 (1963).
Currier, H. B., *Plant Physiol.* **24,** 601–9 (1949).
Hatfield, I., Van Allen, R. G., *J. Amer. Wood Preservers Assoc.* **54,** 23–33 (1958).
Jaworski, E. G., Proc. 10th Ann. Cotton Defol. Conf. pp. 36–40 (1955).
Young, H. C., Carroll, J. C., *Agron. J.* **43,** 504–7 (1951).

Received for review August 16, 1968. Accepted August 6, 1969. Contribution of the Texas Agricultural Experiment Station, Technical Article No. TA 7465. Research sponsored in part by the National Institutes of Health (R G. EF 202) and by the Cotton Research Committee of Texas.