Fate of the Fungicide, 2,6-Dichloro-4-nitroaniline (DCNA) in Plants and Soils

Kermit Groves* and Kwang Suk Chough

The fungicide, 2,6-dichloro-4-nitroaniline (DCNA), was found to be absorbed by plant roots and transformed into natural plant constituents. After incubation of the soils with added DCNA, the rate of decomposition of the fungicide in the soils into carbon dioxide and other products increased. Pure cultures of rod-shaped bacteria that decomposed DCNA were isolated from the soils.

Roburn (1961) reported that 2,6-dichloro-4-nitroaniline, DCNA (Botran, the Upjohn Co.) was not absorbed by plant roots. Lemin (1965) found that the fungicide was absorbed by tomato and lettuce plants after root treatment, and after several weeks very little DCNA remained in the plants; labeled carbohydrates were found after application of labeled DCNA.

The investigations reported here are concerned with the transformations of DCNA in plants and soils.

APPARATUS AND MATERIALS

Chlorine-36 labeled DCNA (Groves and Robbins, 1965) was prepared with an activity of about 500 dpm per μg, and carbon-14 labeled DCNA (Groves, 1967) prepared with an activity of 12,000 dpm per μg. Samples were counted in a Packard Model 3314 Tri-Carb liquid scintillation counter, and the counts converted to decompositions from the efficiencies obtained after spiking the samples with standard toluene-^{14}C. Gas, column, and thin-layer chromatography were used to isolate and determine the fungicide, and the modified colorimetric method of Kilgore and Cheng (1962), and a colorimetric method developed during this investigation based on dye coupling were used (Groves and Chough, 1966).

The evolution of carbon dioxide from soil samples was measured with apparatus consisting of, in order of air flow direction, a carbon dioxide absorption tube, a water saturation bottle, the sample in a bottle, and a Vigreaux type absorption tube containing the hydroxide of Hyamine-10-X for absorbing the carbon dioxide. Air was drawn through the apparatus slowly.

DCNA IN PLANTS

In this laboratory it was found that DCNA was absorbed by the roots of many plants, including bean, corn, oats, lettuce, and tomato. Bean plants were grown in DCNA treated nutrient solution, extracted with benzene, and the extracts purified on a Florosil column. This was followed by purification by preparative gas chromatography. A glass column 1200 mm long, 4 mm i.d., filled with Carbowax 20M, 5% on Anakrom ABS, was used. The temperature of the column, injector, and thermal conductivity detector was 198° C, and the helium flow was 40 ml per min. The infrared spectra of the purified material was identical to that of Eastman Kodak No. 1033 2,6-dichloro-4-nitroaniline.

More DCNA was absorbed by plants from nutrient solutions than from soils under similar conditions (Groves, 1965). The absorption of DCNA from soils by oats (duplicate determinations by glc) was inversely related to the clay and organic matter content of the soils, as shown in Table I. The extraction of DCNA from soils with high clay and organic matter content with organic solvents is more difficult than the recovery from soils containing less of these constituents. The results suggest a binding of DCNA by clay and/or organic matter, the extractability paralleling the uptake of the fungicide by plants.

We found, as had Lemin (1965), that benzene extractable DCNA-^{14}C from root treated plants decreased with time after treatment. Several bean seedlings were grown 48 hr in nutrient solution containing the labeled DCNA, the roots were rinsed, and the plants grown in plain nutrient. Immediately, and at time intervals, plants were extracted with benzene and the extracts counted. The benzene extractable activity decreased with time. The extracted plants were dried and portions burned in oxygen in a Schoniger flask. The labeled carbon dioxide, absorbed in Hyamine-10-X hydroxide, was counted. The total activity in the plants remained constant during the growth period, but the activity changed from benzene extractable to nonextractable. After 4 weeks very little DCNA remained in the plants. The identity of the activity as DCNA was determined by chromatography. The solubility of DCNA in benzene was found to be about 6.6 mg per ml. The solubility in water was about 7 μg per ml. This was determined by saturating water with pure DCNA, and extracting an aliquot with benzene. DCNA partitions into the benzene almost completely. The benzene extract was analyzed colorimetrically and by gas chromatography.

An 11-day-old bean seedling was root treated with DCNA-^{14}C for 2 days, and 212 μg of DCNA was absorbed (by difference). The roots were rinsed and the plant grown in plain nutrient solution under a bell jar, flushed with air. The effluent gases were passed through KOH solutions. Periodically the KOH solutions were treated with barium chloride, and the barium carbonate formed was treated with acid, and the carbon dioxide transferred to Hyamine-10-X hydroxide and counted. After 51 days the whole plant was sampled, including the washed roots. It was extracted with acetone and fractionated, as shown in Figure 1. The observed activities in the fractions are expressed as μg of DCNA. About 88% of the initial activity absorbed by the plant was recovered in the form of natural plant constituents, while only about 1% of the activity was in the form of DCNA.

DCNA IN SOILS

Local soil, previously untreated with DCNA, did not evolve labeled carbon dioxide after the addition of labeled DCNA, and for some time there was no evidence that decomposition in soils occurred. DCNA is strongly absorbed by clay soils, including steam sterilized soils, and is not re-

Department of Agricultural Chemistry, Washington State University, Pullman, Wash. 99163
* To whom correspondence should be addressed.

Table I. DCNA Residues in Oats Grown in Different Soils

Soils, Silt Loams	% Clay	% Organic Matter	DCNA Residue PPM 12 Days after Treatment
Palouse	24.4	4.1	3.0
Western Washington	12.8	6.3	3.3
Ritzville	7.6	1.7	8.7
Warden	3.6	1.7	10.9

Table II. Decomposition of DCNA by Soil-derived Bacillus

Time (days)	Benzene Extractable DCNA (μg)
0	195
1	173
2	182
3	164
4	45
5	0

covered completely by extraction with organic solvents. The apparent disappearance of DCNA from such soils could be attributed to incomplete recovery by extraction. However, the failure of DCNA to control white rot (*Sclerotium cepivorum*) in onion fields in 1967 led to the examination of these soils to which DCNA had been applied for some years with successful control of the fungus. Labeled carbon dioxide was evolved from these soils after application of DCNA-^{14}C. Incubating the soils with DCNA for several weeks increased the rapidity of the decomposition of the fungicide in the soils. Sterilization of the soils by steam, sodium azide, silver nitrate, or mercuric chloride inhibited the decomposition completely.

Extraction of the active soils with water and sodium chloride solutions in an effort to recover active extracellular enzymes resulted in active solutions. However, these apparent solutions were inactivated by high speed centrifuging for 10 min at above 50,000 × g, and it was observed that the centrifuging removed some fine suspended clay particles. Apparently the active microorganisms were associated with fine clay particles, a not uncommon occurrence. Shaking active soils with water, then centrifuging at 1500 × g for 5 to 10 min removed the coarse material, while the supernatant had activities comparable to the original soil. These suspensions were easier to handle in experiments than soil samples. Cultures were made from such suspensions and examined for activity. A culture of rod-shaped bacteria was isolated and found active in the decomposition of DCNA.

The bacteria cultures to which DCNA-^{14}C had been added were examined in the apparatus described above for evolution of carbon dioxide. Between 25 and 50% of the added activity was recovered as labeled carbon dioxide. However, the nonvolatile activity remaining in the culture was not extractable with benzene, indicating that all the DCNA had been altered.

Several Erlenmeyer flasks were prepared, each with 10 ml of nutrient solution and 200 μg of DCNA. Two ml of an aqueous suspension of the bacteria (estimated, 80 million

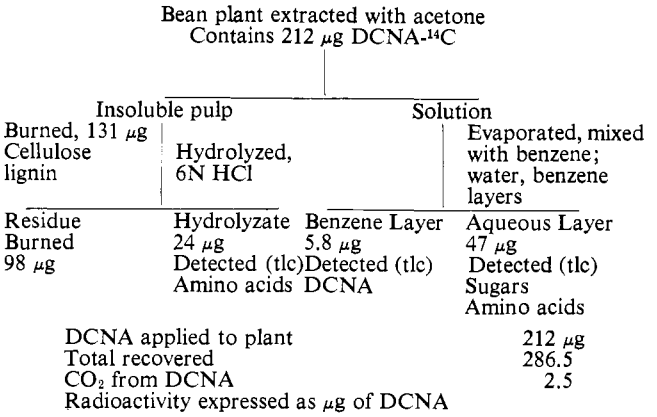

Figure 1. Fate of DCNA in Bean Plant

bacteria) were added to each flask. Each day the contents of a flask were extracted with benzene, and the DCNA in the extracts determined by gas chromatography. The DCNA content of the flasks dropped rapidly to 0 in 5 days, as shown in Table II. The residues in the cultures are being examined for labeled compounds.

Since only part of the decomposed DCNA could be accounted for by the evolved carbon dioxide, other volatile compounds might be evolved. This possibility was examined by passing the air swept through the culture bottle through a combustion furnace containing cupric oxide at 800° to 900° C and passing the exit gases through an absorber. The yield of activity was similar to that obtained by direct absorption in hydroxide of hyamine-10-X. Apparently most of the evolved gaseous activity was in the form of carbon dioxide.

DISCUSSION

Most of the DCNA absorbed by plant roots was accounted for as finally converted into natural plant constituents and a trace of carbon dioxide. Possibly the latter compound served as an intermediate in the formation of natural constituents.

The material balance for soil applied DCNA is not so completely determined. Future work may disclose that the DCNA carbon, not evolved as carbon dioxide, has been incorporated as constituents of the carcasses of microorganisms. Furthermore, the isolated bacillus may be only one of many microorganisms that decompose DCNA cooperatively or independently.

LITERATURE CITED

Groves, K., J. Agr. Food Chem. **15,** 547 (1967).
Groves, K., Paper given before Division of Agricultural and Food Chemistry, 149th National Meeting, ACS, Detroit, Mich., April 7, 1965.
Groves, K., Chough, K. S., J. Agr. Food Chem. **14,** 668 (1966).
Groves, K., Robbins, A. L., J. Agr. Food Chem. **13,** 118 (1965).
Kilgore, W. W., Cheng, K. W., J. Agr. Food Chem. **10,** 399 (1962).
Lemin, A. J., J. Agr. Food Chem. **13,** 557 (1965).
Roburn, J., *J. Sci. Food Agr.* **12,** 766 (1961).

Received for review May 27, 1970. Accepted August 24, 1970. Investigation supported in part by funds provided by the Western Regional Research Project W-45. Scientific Paper No. 3471, College of Agriculture, Washington State University. Work conducted under Project No. 1634.