Efflux of Dichlobenil from Shoots and Roots of Corn

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The root uptake, distribution, and vapor loss of 2,6-dichlorobenzonitrile-7 ^{14}C (dichlobenil) in corn (*Zea mays*) seedlings were determined by assay of plant extracts and vapor trapping solutions. When the concentration of ^{14}C-dichlobenil in the nutrient solution was maintained at approximately 0.3 p.p.m., the maximum quantity in the corn roots was attained after 12 hours and the quantity in the shoots continued to increase up to 72 hours. Unaltered ^{14}C-dichlobenil vapor was emitted from the shoots of preloaded corn plants similarly in the light and dark, but the efflux was temperature dependent. The quantity of herbicide emitted from plants in 24 hours was 70 to 80% of the amount absorbed. Root loss was sevenfold the amount lost from shoots over a similar 24-hour trapping period at 27° C.

The herbicidal properties of dichlobenil (2,6-dichlorobenzonitrile) were first described by Koopman and Daams (1960). This compound is particularly toxic to meristematic tissues and strongly inhibits the growth of germinating seedlings. Dichlobenil effectively controls many annual and certain perennial weeds in both orchards and nurseries.

Dichlobenil is a relatively volatile compound with a vapor pressure of 5.5×10^{-4} mm. of Hg at 20° C. Pate and Funderburk (1966) determined that nearly 98% of the radioactivity from ^{14}C-dichlobenil was lost from an open planchet in 2 hours.

Dichlobenil is absorbed only slightly when applied as a foliar spray; however, it is readily absorbed by the roots (Pate and Funderburk, 1966). It is translocated throughout the plant via the xylem, but more slowly than water owing to its strong affinity to plant tissue (Mazzini, 1961). When bean plants were exposed to a saturated atmosphere of dichlobenil vapor, the compound was absorbed by all aerial parts. Verloop and Nimmo (1966) found that the concentration of dichlobenil in bean leaves decreased from about one-fourth (after 1 day) to about 1/20 (after 5 days) of the root concentration. They postulated that the decrease may be caused by two phenomena: the herbicide evaporates out of the leaves, or it is degraded by plants.

A water-soluble metabolite of dichlobenil was detected in bean, alligator weed, and four soil fungi and identified as 2,6-dichlorobenzoic acid (Pate and Funderburk, 1966). Mazzini (1961) also presented evidence that dichlobenil is metabolized in plants.

Preliminary experiments in this laboratory indicated that a significant loss of radioactivity occurred from corn plants after 72 hours of exposure. The objective of this research was to determine if unaltered ^{14}C-dichlobenil was lost directly from plants as vapor.

MATERIALS AND METHODS

Preparation of Plant Material. Corn (*Zea mays*, "Harris Gold Cup") seeds were germinated in vermiculite wetted with distilled water. After the first leaf emerged from the coleoptile, the seedlings were removed and transplanted into quartz sand. The pots were watered daily with one-half strength Hoagland's solution and maintained in a growth chamber with a 16-hour photoperiod at 27° C. and an 8-hour night at 21° C. When the plants were approximately 6 inches tall, they were removed from the sand and the seed coats excised. After thorough rinsing of the roots, the plants were placed in 250-ml. beakers containing 200 ml. of Hoagland's solution. Plants were allowed to equilibrate in this aerated nutrient solution 24 hours prior to treatment.

Uptake of dichlobenil was studied by replacing the nutrient solution in the beakers of the plants to be treated with nutrient solution containing 1.0 μc. of nitrile-labeled ^{14}C-dichlobenil (specific activity 4.65 mc. per mmole) per 100 ml. of solution. The initial concentration of dichlobenil in the treating solution was 0.37 p.p.m. Owing to the volatility of this compound, all studies were carried out in the hood under a bank of fluorescent lights. After 4, 12, 36, and 72 hours, plants were removed from the radioactive solution and extracted for assay. Three to four replicates were assayed in each experiment and all experiments were duplicated at least twice. Aliquots from the nutrient solution were also analyzed for radioactivity at each sampling. This was accomplished by bringing the nutrient solution to a constant volume and removing 1.0 ml. which was placed in a scintillation vial containing 15 ml. of toluene BBOT [2,5-bis(5-*tert*-butylbenzoxazolyl)] thiophene–Triton X-100 (10 to 4). The radioactivity in the solution was determined by liquid scintillation spectrometry.

Vapor Trapping. To study the efflux of dichlobenil from the leaves and roots of corn plants, 12 plants were preloaded with ^{14}C-dichlobenil for 24 hours. After rinsing the roots six times in distilled water, six plants were immediately extracted and assayed for radioactivity. The remainder of the plants were placed in three 25-ml. Erlenmeyer flasks containing 20 ml. of fresh nutrient solution. The top of each flask was sealed with a plug of cotton and polyethylene glycol 1500. Control flasks were prepared with 20 ml. of nutrient solution containing 0.05 μc. of ^{14}C-dichlobenil and stoppered similarly. These flasks were also placed within the trapping device (Figure 1) to assure that no radioactivity from the nutrient solution entered the system. Glass tubes were cemented into the metal tops of the Mason jar chambers to provide an air inlet and outlet. To the outlet tube, two 200-ml. gas washing bottles with glass fritted disks were connected in series with short sections of Tygon tubing. Rubber could not be used because of its high affinity for dichlobenil.

The gas washing bottles were filled with 100 ml. of toluene as a trapping solution and were maintained in an

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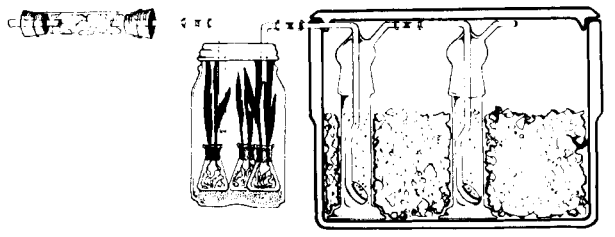

Figure 1. Apparatus for trapping ^{14}C-dichlobenil volatilized from corn leaves

ice bath. Filtered, compressed air was passed through a $CaCl_2$ moisture trap into the Mason jar containing the plants and was bubbled through the toluene trapping solutions for 24 hours.

The trapping solutions were brought to a constant volume, and 20-ml. aliquots were removed for assay. Eighty milligrams of BBOT were added to this fraction for counting and the remainder of the solution was condensed in a vacuum evaporator for the identification. The nutrient solution was also assayed for radioactivity at the end of the trapping period to detect dichlobenil emitted from the roots.

Extraction and Identification. Plants for assay were removed from the nutrient solution, after which the roots were rinsed five times with distilled water, blotted, and dissected from the shoots. The samples were cut into small sections with a razor blade and quick frozen in dry ice. The frozen samples were weighed and subsequently extracted with 4 ml. of benzene in a glass homogenizer. The homogenate was vacuum filtered through Whatman No. 1 filter paper and the residue rinsed three times with benzene. The filtrate was brought up to a constant volume, and a 1.0-ml. aliquot removed for assay. The plant residue was also assayed for radioactivity. After counting, the data were corrected for quenching and the results expressed as disintegrations per minute per milligram of fresh weight.

The radioactivity in plant extracts, trapping solutions, and nutrient solutions was identified by thin-layer cochromatography with ^{14}C-dichlobenil on silica gel G plates utilizing the solvent systems of ethanol-hexane, 1-to-9 v./v. (Beynon *et al.*, 1966) and diethyl ether–petroleum ether, 1-to-1 v./v. at 3° C. (Pate and Funderburk, 1966). Twenty microliters of the condensed sample were spotted on the thin-layer plate. After ascending development, the chromatograms were cut into 1-cm. sections and placed in vials for liquid scintillation assay.

RESULTS AND DISCUSSION

In preliminary experiments, using the extraction procedure of Meulemans and Upton (1966), no water-soluble metabolites of dichlobenil were found in corn plants or nutrient solutions after 72 hours, indicating that a single benzene extraction would be sufficient to account for essentially all of the radioactivity.

Experiments on the uptake of ^{14}C-dichlobenil by corn plants from an aerated nutrient solution demonstrated that 57% of the radioactivity was lost from the nutrient solution within 12 hours (Figure 2). The loss of radioactivity was not accounted for by the amount taken up by the plants, which suggested a loss directly from the nutrient solution. A loss of this magnitude would be recognized as an important factor in experiments where the concentration of dichlobenil is being studied. Also, if special precautions are not taken, escaping vapors may cause contamination of adjacent plants and equipment.

With a rapid aeration system (100 ml. of air per minute) the highest concentration of ^{14}C-dichlobenil in the roots was attained at 4 hours and then continuously decreased until the experiment was terminated at 72 hours (Figure 3). The decrease in ^{14}C-dichlobenil concentration in the roots closely paralleled the decrease of ^{14}C-dichlobenil in the nutrient solution. The concentration of ^{14}C-dichlobenil in the shoot continued to increase for 36 hours but decreased over the subsequent 36 hours, indicating that with a limited source of ^{14}C-dichlobenil there was either a net loss from the shoot due to volatilization from the leaves, recycling and loss from the roots, or metabolism of the parent compound.

A relatively constant concentration of ^{14}C-dichlobenil in the nutrient solution was attained by reducing the aera-

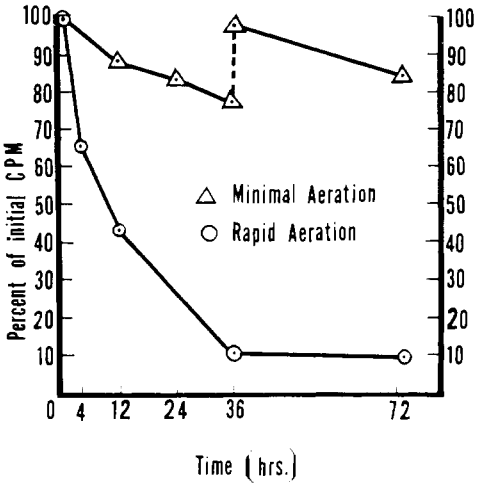

Figure 2. Per cent of initial CPM in nutrient solution, using minimal and rapid aeration

At 36 hours minimal aeration scheme was brought up to the original concentration by adding sufficient ^{14}C-dichlobenil

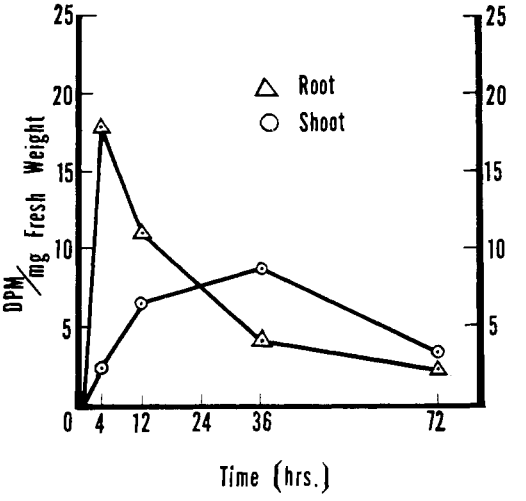

Figure 3. Radioactivity recovered from roots and shoots of corn plants grown in rapidly aerated nutrient solution containing ^{14}C-dichlobenil

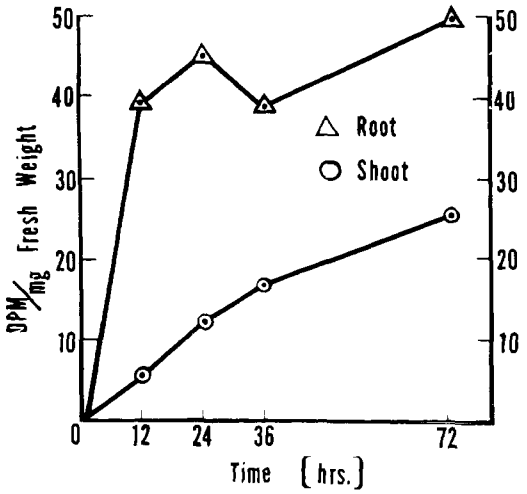

Figure 4. Radioactivity recovered from roots and shoots of corn plants grown in nutrient solution containing ^{14}C-dichlobenil with minimal aeration

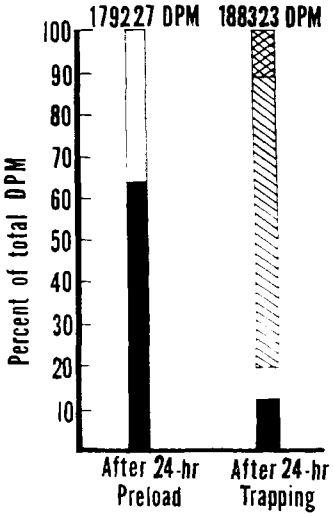

Figure 5. Distribution of radioactivity in corn plants after preloading with ^{14}C-dichlobenil for 24 hours and after 24-hour vapor trapping period

Shoot, Root, Nutrient solution, Toluene traps,

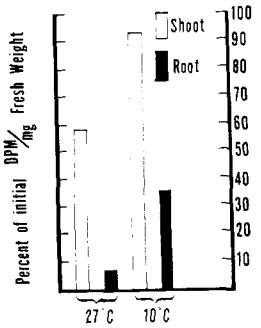

Figure 6. Per cent of initial radioactivity remaining in preloaded corn plants after exposure to 27° and 10° C. for 24-hour loss period

tion to a minimum (15 ml. of air per minute) and adding sufficient ^{14}C-dichlobenil after 36 hours to return the nutrient solution to its original concentration (Figure 2). Utilizing this procedure, both the root and shoot contained increasing amounts of radioactivity over the 72-hour period (Figure 4). Under these conditions, any loss of ^{14}C-compounds from the shoots was masked by continued uptake.

In the vapor trapping experiments, 9.9% of the initial radioactivity present in the shoots was collected in the toluene traps after 24 hours (Figure 5). The R_f for the radioactivity in the condensed trapping solution was identical to that of ^{14}C-dichlobenil on silica gel plates. With ethanol-hexane (1 to 9) the R_f was 0.4 and with diethyl ether–petroleum ether (1 to 1) the R_f was 0.6.

The largest quantity (70.8%) of radioactivity was detected in the nutrient solution, indicating rapid diffusion from the roots with a concentration gradient. Dichlobenil apparently is not actively held within the root cells, but readily passes through intervening membranes and cell walls. Since the amount lost to the nutrient solution plus that recovered from the roots was greater than the initial quantity in the roots after preloading, there is probably a cycling of ^{14}C-dichlobenil from the shoot to the root. The total amount of radioactivity recovered after 24 hours was within 5% of the initial radioactivity recovered in the plants before trapping was initiated. No radioactivity was detected in the chambers with stoppered control flasks containing only ^{14}C-dichlobenil in the nutrient solution.

The loss of ^{14}C-dichlobenil from preloaded corn plants was similar in light and darkness at 27° C. At 10° C. there was very little loss of radioactivity from the shoot; however, at 27° C. the shoot contained only 58.2% of the initial activity (Figure 6). Similarly, the efflux from the roots was greater at the higher temperature, although a much greater loss also occurred from roots than shoots at 10° C.

The data indicate that dichlobenil readily penetrates most plant tissues and is not actively held within cells. Since dichlobenil does have a high vapor pressure, it is feasible that under the proper temperature conditions, it would vaporize either within the leaf or from the surface of the leaf. The fact that vapor loss occurs at the same rate in both the light and dark lends some evidence that the loss is cuticular. However, evidence is not conclusive since the condition of the stomata was not determined. The lipophilic nature of the dichlobenil molecule may explain the apparent ease of movement through plasmalemma and cuticular membranes. To our knowledge, this is the first published report of uptake, translocation, and subsequent vapor loss of an unaltered pesticide from aerial portions of intact plants.

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