Enhanced Absorption and Persistence of Carbofuran and Chlordimeform in Rice Plant on Root Zone Application Under Flooded Conditions

G. B. Aquino and M. D. Pathak
The International Rice Research Institute, College, Laguna, Philippines

ABSTRACT

The effect of 4 methods of application of chlordimeform and carbofuran on the uptake and persistence of their residues in rice plants was compared. The highest concentration of the residues of 2 insecticides in roots, stem, and leaf blade occurred when the insecticide, in gelatin capsules, was placed in the root zone of each hill of rice. The persistence of the insecticide residues in plant tissues was longer when the insecticide was incorporated into the top soil than when the compound was applied to the paddy water. Placement of the insecticide in the root zone in the center of 4 hills delayed absorption and translocation of the residues of chlordimeform and carbofuran.

The use of foliar insecticidal sprays generally has been ineffective against most of the common rice pests in the tropics where overlapping generations of such pests are common and where rains frequently wash the insecticides from the plants. These problems were largely overcome by applying certain insecticides to the paddy water, soil surface or incorporating them in the top soil of the paddy fields (Koshihara and Okamoto 1957, Kawada 1967, Rao and Israel 1967, Pathak 1967, 1968, Pathak et al. 1971). When thus applied these insecticides are usually effective for 20–30 days as compared to 7–10 days effectiveness of most foliar sprays. Thus, this method of application has become popular in Asia and has been investigated extensively (Fukuda 1968, Lilly et al. 1970, Lee 1965, Koyama 1971, Iwata 1973, Pathak and Dyck 1973). Bowling (1970) obtained similar results in the United States with carbofuran which in laboratory experiments caused nearly 100% mortality of a leafhopper, Draeculacephala portola Ball, caged on rice plants 2 h after insecticidal application.

Other methods of insecticidal applications have been investigated, such as seed treatment, seedbed treatment, and treating the roots of rice seedlings prior to their transplanting. These methods are generally effective only for brief periods and need to be supplemented with other treatments in most of Asia where the rice crop is subjected to infestation by many insect pest species. Recent studies at the International Rice Research Institute have demonstrated that certain insecticides, placed in a capsule or used as a large granule, inserted ca. 2.5-cm below the soil surface near the base of a rice plant provide insect control for 80–100 days after treatment (Pathak et al. 1974). This usually obviates the need for another insecticidal treatment. However, when the insecticide is applied to the paddy water, a reaplication is needed every 20–30 days to ensure adequate plant protection.

We compared the influence of 4 methods of application on the levels of carbofuran and chlordimeform residues in the rice plant and the results are reported here.

MATERIALS AND METHODS.—Field Experiment.—Fields were thoroughly puddled, followed by a basal application of 60 kg N/ha. Rice seedlings (21 days old) were transplanted with a spacing of 25 cm between plants. An additional 30 kg N/ha was applied as a top dressing 50–60 days after transplanting.

IR22 rice plants were transplanted on May 6, 1973, in a 44 m² plot. The chlordimeform granules were applied at 2.0–2.12 kg AI/ha. The 4 treatments were replicated 4 times.

IR20 rice seedlings were transplanted on Dec. 1, 1973, in a 34.85 sq m plot. Carbofuran was applied at 2 kg AI/ha. The 4 treatments were replicated 3 times.

The treatments were: (i) 12.35 mg AI insecticide in a gelatin capsule placed 2 to 3 cm below the soil and 2–3 cm laterally from each hill 2 days after transplanting; (ii) 49.4 mg AI insecticide in a gelatin capsule placed 2–3 cm below the soil at the center of 4 hills 2 days after transplanting; (iii) granular insecticides were incorporated into the top soil just before transplanting the crop; (iv) granular insecticides were applied to the paddy water 1 to 2 days after transplanting; and (v) untreated control.

All test plots were separated by mud levees and all insecticidal treatment were applied only once. Because of the specific gravity of the special granules supplied by the manufacturer, chlordimeform was applied at 13.25 mg AI in treatment (i) and at 53.0 mg AI/ha in treatment (ii). The remaining treatments were applied at 2.0 kg of the insecticides/ha. The water depth was maintained at 5 cm in all plots throughout the experiment. All other standard agronomic practices were followed.

Sample Collection.—At 5 premarked locations in each plot, a 100-ml water sample was collected, and these were pooled for residue analysis. Depending on the age of the rice plants, 10–20 randomly selected rice hills constituted a sample from 1 plot. These were separated into roots, stem, and leaf blade;
chopped; and thoroughly mixed. Both the water and the plant samples were stored at \(-10^\circ C\) until analyzed.

**Analytical methods.---Chlordimeform in Plant Tissue.**--Chopped plant samples (20-25 g) were hydrolyzed with acetic acid, followed by sodium hydroxide, to 4-chloro-o-toluidine, which was diazotized. The diazo moiety was then exchanged for iodine by treatment with potassium iodide (Geissbühler et al. 1971). The iodinated derivative was determined by electron capture gas chromatography. Plant samples fortified at 0.1 ppm showed good recovery (90%).

The iodinated derivative was analyzed on a Varian Aerograph model 204B fitted with a H\(^+\) electron capture detector. A 5 ft \(\times\) 1/4 in. borosilicate glass column was packed with 3% silicon SF 96 on Gas Chrom Q 80-100 mesh (Varian Aerograph). The injection port temperature was 150°C, while that for the column was 120°C. The temperature of the detector was 200°C and cell voltage was fixed at -90 volts DC. The flow rate of the nitrogen carrier gas was 28-30 ml/min. Under these conditions, the iodinated derivative had a retention time of 2.38 min.

**Carbofuran in Paddy Water.**--Paddy water (50 ml) was extracted 3 times with 50 ml methylene chloride in a 250 ml separatory funnel by shaking manually for one minute. The organic phase was washed quickly twice with 50 ml of cold 0.25 N sodium hydroxide. The organic fraction was dried with the addition of anhydrous sodium sulfate. The extract was concentrated to ca. 1 ml in a rotary evaporator.

The carbofuran content was determined by reaction of the residue on a steam bath for 30 min with 0.5 ml of 1% 1-fluoro 2,4-dinitrobenzene in 25 ml pH 9 borax buffer solution and 50 ml acetone (Fullmer 1974). The 2,4-dinitrophenyl ether produced in this reaction was determined by gas chromatography after extraction in hexane. Plant samples were fortified at 0.2 ppm and had a carbofuran recovery of ca. 90%.

The 2,4-dinitrophenyl ether of carbofuran in hexane extract was analyzed by gas liquid chromatography in a Varian Aerograph Model 1700 equipped with a Ni\(^2+\) electron capture detector. The spiral borosilicate glass column (7 ft \(\times\) 1/4 in. od) was packed with 5% DC200 silicone on Gas Chrom Q, 80-100 mesh. The temperature of the injector port was 245°C, while that of the column was 215°C. The temperature of the detector was 265°C. The flow rate of the nitrogen carrier gas was 67 ml/min. Under these conditions, the carbofuran derivative had a retention time of 1.2 min. The observed residue value was corrected according to the ratio between the molecular weight of carbofuran and that of the derivatized 2,4-dinitrophenyl ether.

**Carbofuran in Plant Tissue.**--Chopped plant samples (15-25 g) were refluxed with 150-250 ml of cold 0.25 N HCl for one hour with continuous stirring (Cook et al. 1969). An additional 100 ml were used to wash the reflux flask and condenser, and 200-300 ml were filtered and cooled for one hour at \(-10^\circ C\) before partitioning into methylene chloride by shaking the filtrate with three 50-ml portions of the solvent in a separatory funnel. A 10% solution of sodium lauryl sulfate in water was added to prevent emulsification. The methylene chloride layers were combined, dried with sodium sulfate, and concentrated to ca. one ml by a rotary evaporator under vacuum.

Clean-up and separation of the carbamate from the phenols were carried out in a column of 10 g of adsorption alumina (Bowman and Beroza 1967). The carbamates were eluted with 100 ml of redistilled chloroform.

The carbamate content was determined quantitatively by evaporating the chloroform extract to near dryness, and following those procedures, used in the analyses of the paddy water samples. Further clean-up of some of the samples with a one-gram nuclear G190N (Fisher Scientific Co.) column was carried out to resolve the interfering peaks, with 60 ml of methylene chloride serving as an eluting solvent. Fortification tests carried through the entire procedure showed good recovery of 88%.

**RESULTS AND DISCUSSION.**--Residue in Standing Water.---The lowest level of carbofuran during the 1st 10 days was recorded in the treatment in which the insecticide was placed in the root zone of rice plants (Table I). The highest level of residue in standing water occurred in those plots where the insecticide granules had been broadcast on the soil surface. This was expected since the carbofuran on the soil surface was in direct contact with the standing water. The low concentration of insecticide in the paddy water in those plots where it had been applied to the root zone is highly desirable because it would be less hazardous to fish in paddy fields and would provide less contamination to the other sources of water. Several species of fish exposed to various concentrations of carbofuran formulations for 96 h were able to tolerate higher levels of carbofuran (0.21-4.1 ppm) than DDT (0.01 to 0.05 ppm) (Anon. 1970) and exhibited a median tolerance for carbofuran which was higher than the concentrations observed in the present study. This suggests that fish

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carbofuran concentration in paddy water (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td>Root zone application</td>
<td></td>
</tr>
<tr>
<td>Each hill</td>
<td>0.016</td>
</tr>
<tr>
<td>Center of 4 hills</td>
<td>0.004</td>
</tr>
<tr>
<td>Paddy water application</td>
<td>0.206</td>
</tr>
<tr>
<td>Incorporation in top soil</td>
<td>0.055</td>
</tr>
<tr>
<td>Control</td>
<td>0.006</td>
</tr>
</tbody>
</table>

* DT = days after transplanting; trace = less than 0.003 ppm.

---

* Fullmer, O. H. 1974. Personal communication, FMC Corporation, Agricultural Chemical Division, Richmond, Calif. 94804.
Table 2.—Effect of the method of application on the concentration of carbofuran in the roots of IR20 rice plants, IRRI, 1974.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>5 DT*</th>
<th>10 DT</th>
<th>20 DT</th>
<th>40 DT</th>
<th>80 DT</th>
<th>100 DT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root zone application</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Each hill</td>
<td>0.535</td>
<td>0.764</td>
<td>1.605</td>
<td>0.312</td>
<td>0.031</td>
<td>0.010</td>
</tr>
<tr>
<td>Center of 4 hills</td>
<td>0.009</td>
<td>0.009</td>
<td>0.015</td>
<td>0.016</td>
<td>0.038</td>
<td>0.015</td>
</tr>
<tr>
<td>Paddy water application</td>
<td>0.186</td>
<td>0.102</td>
<td>0.071</td>
<td>0.007</td>
<td>0.008</td>
<td>trace</td>
</tr>
<tr>
<td>Incorporation in top soil</td>
<td>0.277</td>
<td>0.230</td>
<td>0.239</td>
<td>0.058</td>
<td>trace</td>
<td>trace</td>
</tr>
<tr>
<td>Control</td>
<td>0.015</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
<td>trace</td>
</tr>
</tbody>
</table>

* DT = days after transplanting; trace = less than 0.003 ppm.

could be cultured in rice paddies treated in the root zone area with carbofuran.

Residue in Plant Tissues.—Both chlordimeform and carbofuran were readily absorbed by the roots and translocated to the shoots in all treatments. However, the concentration of the insecticides was highest where each hill received the root zone application (Table 2 and Fig. 1), because the roots are in direct contact with the insecticide. The minimum concentration of insecticide in plant tissue occurred during the 1st 20 days after the insecticides were placed at the center of 4 hills. However, the concentration increased ca. 40 days after application, indicating that the plant roots reached the site where the insecticide was placed. Probably the roots of the plant during the 1st 20 days did not have access to the insecticide and the amount absorbed by the plant may be attributed to the lateral movement of the insecticide in the soil and water. Bowling (1970) recorded that carbofuran applied to flooded rice moved laterally 22.5 cm in 48 h in quantities toxic to leafhoppers. This fact is critical because it emphasizes the importance of placement as a factor that influences the absorption and translocation of insecticides in rice plants.

The concentration of both insecticides in stem and leaf blade tissue was highest in plants from plots where the insecticide had been placed in the root zone of the individual hills. A maximum concentration of ca. 44.5 ppm chlordimeform and its metabolites was recorded in shoots 5 days after application (Fig. 2) and 9 ppm at 40 days after treatment, as compared with the highest concentration of only 4 ppm at 5 days after application of the insecticide to the paddy water.

At present, the paddy water method of application is the most effective method used for pest control in paddy rice. Thus, the significance of the effectiveness of applying an insecticide at the root zone is obvious.

The pattern of absorption and translocation was similar in plots treated with carbofuran; the concentration was greater in the leaf blades than in the stem in all treatments (Table 3). A maximum concentration of ca. 69 ppm of carbofuran in plant shoots occurred where the insecticide was applied to the root zone; more than 90% were concentrated in the leaf blade. A high concentration of the insecticide in the leaf blade is significant for plant protection, because *Hydrellia philippina* Ferino and leafhoppers, *Nephotettix* spp., initiate feeding on the leaf blade of the rice plant. The concentration of the insecticide in plant shoots 40 days after treatment was ca. 7 times greater when the insecticide was placed in the root zone as compared with the broadcast application.

The persistence of both insecticides in plant tissue was greater when the compound was incorporated into the soil than when it was only broadcast on the soil surface. The concentration of chlordimeform in the tissue 40 days after treatment of rice plants grown in plots where the insecticide was incorporated into the soil was comparable to that at 10 days when the insecticide was applied to the paddy water. Simi-
Table 3.-Effect of the method of application on the concentration of carbofuran in the leaf blade of IR20 rice plants, IRRI, 1974.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Carbofuran concentration in the leaf blade (μg/g fresh wt basis)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 DT</td>
</tr>
<tr>
<td>Root zone application</td>
<td></td>
</tr>
<tr>
<td>Each hill</td>
<td>16.571</td>
</tr>
<tr>
<td>Center of 4 hills</td>
<td>0.017</td>
</tr>
<tr>
<td>Paddy water application</td>
<td>0.523</td>
</tr>
<tr>
<td>Incorporation in top soil</td>
<td>2.590</td>
</tr>
<tr>
<td>Control</td>
<td>0.050</td>
</tr>
</tbody>
</table>

*DT = days after treatment; trace = less than 0.003 ppm.
*The carbofuran contents in plant stems from these 2 treatments at 10 DT were 2.257 and 0.576 μg/g, respectively, and followed the same pattern of decline as in leaf blades. They were very low in other treatments.

insects for a longer time than when it is broadcast to the paddy water. A single application of an insecticide to the root zone of a rice plant was found to be comparable to 3-4 applications of the chemical to paddy water (Pathak et al. 1974). Additionally, incorporation of an insecticide into the top soil affords greater crop protection than applying the compound to the paddy water because its residue has a long persistence in the plant tissue. Apparently, the insecticide below the soil surface is available to the roots of the crop. Also, degradation caused by photodecomposition and losses due to physical factors, such as seepage and runoff, are minimized.

Crop Protection.-Placement of insecticides in the root zone of each rice hill or incorporation into the paddy soil significantly reduced the incidence of dead hearts caused by attack of the Asian rice borer, *Chilo suppressalis* (Walker), (Table 4). Carbofuran applied to paddy water was effective in controlling *H. philippina* only during the early stages of crop growth but did not control the Asian rice borer which caused dead hearts. The level of carbofuran residue in the stem and leaf blade of the rice plant during the 1st 20-25 days appeared to effect good control of this insect (Table 3). None of the treatments effectively reduced the incidence of white heads which occur during the later stages of the growth of the rice plant. The low concentration of carbofuran residue in plant tissues at 80 to 100 days after treatment was probably not adequate to kill borer larvae feeding in the stem of the rice plant.

Generally, plots protected from insect damage had significantly higher grain yields than the untreated control. However, the grain yields were significantly higher in treatments where carbofuran was applied to the root zone of each hill or incorporated into the soil prior to transplanting than when the chemical was applied (2 kg AI ha⁻¹) once to the paddy water. The grain yield of plots where the insect-
Cide was placed in the soil among 4 hills and that of the plots where the compound was applied to the paddy water did not differ significantly. The high concentration of carbofuran residue in the tissue of rice plants in plots where the insecticide was placed in the root zone of each hill or incorporated into the top soil contributed significantly towards increasing the grain yield over that of plots where the insecticide was applied to the paddy water. Thus, the advantage of these 2 methods, particularly that of applying the insecticide to the root zone of paddy rice, in protecting the crop from tropical rice pests is clear.

Acknowledgment.—We thank the Niagara Chemical Division of the FMC Corporation, Middleport, N.Y., for providing the carbofuran standard and Schering AG, Berlin, Germany, for furnishing the 4-chloro-o-toluidine standard.

REFERENCES CITED