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Simulated Rainfall Removal of Tricyclazole Sprayed on Rice Foliage

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Abstract Two rainfall simulations of 30 mm h^{-1}, with 48-h interval between two simulations, were performed on rice lysimeters at 24, 48, and 72 h after being sprayed with tricyclazole. In the first simulated rainfall, wash-off concentration of tricyclazole was significant irrespective of the interval between the spray time and the rainfall simulation. And from 20.5% to 24.2% of tricyclazole deposited on leaves was removed from the rice foliage. In the second simulated rainfall, concentration of tricyclazole in wash-off water was significantly lower and less than 3.6% of the deposited tricyclazole was lost.

Keywords Tricyclazole · Rice · Foliar wash-off · Lysimeter

Rainfall can reduce the efficacy of pesticides by washing the foliage-applied active compounds off the plant and also can increase the availability of pesticide for runoff (Reddy et al. 1994). Therefore, rainfall can significantly affect the environmental fate of foliar-applied pesticides. And the elapsed time between pesticide application and the first rainfall event can affect pesticide persistence and wash-off losses. Concentrations and amounts washed from foliage may be a function of the probability of rain occurring within a given time after application (Willis et al. 1994). Information on foliar wash-off is essential for environmental modeling and management practice but most of the wash-off studies were on plants rather than rice. While full-scale field experiments are difficult and time-consuming, simulated rainfall can provide useful data on vulnerability of pesticides to foliar wash-off (Reddy et al. 1994). A combination of rainfall simulation and lysimeter can be a good method to simulate agricultural conditions to study the fate of chemicals, especially when it is scaled down for in-house laboratory experiment.

Tricyclazole (5-methyl-1,2,4-triazolo[3,4-b]benzothiazole) is a common systemic fungicide used to control the rice blast, especially in Asian countries. Although new granular formulations of tricyclazole have been marketed, a significant amount of this fungicide is still sprayed on rice foliage each year because foliage application of tricyclazole is the most effective cure against rice blast (Shiba and Nagata 1981). Tricyclazole is relatively soluble (water solubility: 1,600 mg L^{-1}) thus can be easily washed from rice leaves by rain water. In Europe, the environmental risk of this compound is considered high for surface water (Padovani et al. 2006). However, the behavior of tricyclazole sprayed on rice plants, especially under rainfall condition, has rarely been reported in the literature. Knowledge about the wash-off process in rice plant is also needed for modeling purpose. Therefore, our study was aimed to investigate the behavior of tricyclazole applied to rice foliage and the amount of tricyclazole wash-off under two consecutive simulated rainfall events.

Materials and Methods

Three micro lysimeters made from stainless steel (50 cm × 35 cm × 30 cm L × W × H) were used for this study in

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2007. Rice plants (*Oriza sativa* L. Tsukinohikari) were extracted from the field and washed to remove dirt and soil in the root. Rice plant was then placed into stainless steel baskets (15 cm diameter × 5 cm height) with gravel support so that the plant can stand alone in the lysimeter. Each lysimeter contained four plants to represent the Japanese standard transplanting grid (30 cm × 15 cm). The canopy cover was estimated by using the Photoshop software to analyze pictures taken above the foliage. Rice was hydroponically cultivated until the experiment. Solutions of tricyclazole, prepared by diluting the commercial formulation BEAMzol (Kumiai Chemical Industry, Tokyo, Japan) with water, was sprayed by a previously calibrated garden sprayer.

A drop-former rainfall simulator, which covers the area of the lysimeter, was used to apply 30 mm of rain in 1 h. Return period of a natural rainfall of 30 mm h^{-1} for 1 h is about 1 year in Tokyo, Japan (Tokyo Meteorology Department). The simulator was calibrated at the targeted rate. The average distribution uniformity coefficient of 90.6% was determined, with a deviation of only about 2.0%. Rainfall simulations were initiated 24, 48 and 72 h after tricyclazole application (called set 1, set 2 and set 3). After a 48-h interval from the first rainfall event, a second simulation was again initiated (72, 96, and 120 h after application for set 1, set 2, and set 3, respectively).

Before spraying, 6-cm depth of water was added to the lysimeter to measure the sprayed fungicide that is not intercepted by the rice foliage within the lysimter. The water compartment in lysimeters was sampled and drained completely before rainfall event. In set 1, spray drift outside of the lysimeter was evaluated by placing 4 water sensitive papers (5.2 cm × 7.6 cm) (Syngenta Crop Protection, Basel, Switzerland) around each lysimeter. However, in set 2 and set 3, spray drift was collected by spreading 20-cm wide aluminum foil around the lysimeter. The foil was later washed with water-acetonitrile (80:20) and diluted with the same solvent mixture for chromatographic analysis. During the rainfall simulation, the lysimeter was slightly inclined (4%) so that wash-off water could be collected using both drainage ports at the bottom of the lysimeter and a peristaltic pump at the lower corner of the lysimeter. Samples were collected every 5 mm of rainfall. After simulation, another 6-cm depth of water was added to the lysimeter. Similar procedure was followed in the second simulation.

Water samples as well as drift extracts were filtered through 0.2 μm syringe filters and transferred to 2-mL vial prior to analysis. The water samples were analyzed using an Agilent (Palo Alto, USA) HP1100 series liquid chromatograph linked with a single quadrupole mass spectrometer, Waters Micromass ZQ 4000 (Manchester, UK) utilizing Electron Spray Ionization (ESI) in positive ion mode. The mass spectrometer was set in the selected ion monitoring mode. A Wakosil-II 5C18 AR column (4.6 mm × 150 mm, 5 μm particle size) was used at a flow rate of 0.3 mL min^{-1} and maintained at a temperature of 40°C. The mobile phase was acetonitril-water (80:20, v/v). The detection limit was 0.5 μg L^{-1} and the recovery (n = 3) was 98.2 ± 5.3%.

Results and Discussion

The actual application rates were different among experiment scenarios (Table 1). The variation was caused by the error during weighing a small amount of a viscous pesticide formulation. However, these rates were still in the range recommended by the manufacturer. The canopy cover values and the water collection efficiency during rainfall simulation are also given in Table 1. There were differences in canopy cover values because the rice plants were growing in the field during that time. The efficiency in collecting the simulated rain was high in all replications. The variation was low noting that the size of the lysimeter is small. The uncollected water was assumed to be lost through stem flow, drift, evaporation and plant interception. Wash-off amount was not corrected for water collection efficiency.

The concentration of tricyclazole in the water compartment of the lysimeter after spray and the mass balance of tricyclazole after spray were calculated and given in Table 2. Concentrations of tricyclazole in the water of the lysimeter after spray ranged from 100.5 to 26.7 μg L^{-1}, which are comparable to the measured concentrations of 60–70 μg L^{-1} in field lysimeters (data not shown).

The fraction of spray drifted outside the lysimeter were 7.2% and 12.8% of the applied mass of tricyclazole for set 2 and set 3, respectively. Spray drift in set 1 was only qualitatively evaluated by using water sensitive paper. By comparing the droplet density on the water sensitive paper and on the foil surface, the amount of drift in set 1 was estimated to be in the same range with two other sets. Large variations in drift fraction may be because of the

Table 1 Application rate, canopy cover, and water collection efficiency

	Set 1	Set 2	Set 3
Application rate (g ha^{-1})	241.9	178.3	154.4
Canopy cover (%)	67 ± 1	74 ± 6	75 ± 6
Water collection efficiency (%)			
First rainfall	86.1 ± 4.4	85.6 ± 2.9	88.0 ± 6.3
Second rainfall	86.6 ± 4.9	88.7 ± 2.4	88.9 ± 0.7

Table 2 Water concentration and mass balance of tricyclazole after spray

	Set 1	Set 2	Set 3
Water concentration after spray ($\mu g\ L^{-1}$)	100.5 ± 12.0	55.6 ± 12.3	26.7 ± 5.3
Drift fraction (%)	NA	7.2 ± 1.6	12.8 ± 3.5
Mass fraction of tricyclazole found in water compartment (%)	24.9 ± 3.0	18.7 ± 4.1	10.4 ± 2.1
Mass fraction deposit on plant[a]	65.1 ± 3.0	74.1 ± 2.7	76.8 ± 5.2

[a] For this calculation, the drift fraction of set 1 was assumed to equal the average of set 2 and set 3

small scale of the experiment and of the use of hand sprayer.

The mass of tricyclazole in water decreased from 24.9% to 10.4% of the applied mass over three sets, corresponding to the increase in the mass of tricyclazole deposited on rice plant. It means that the interception of tricyclazole by rice plant was proportional to the canopy cover. This relation was also reported by Wauchope and Street (1987) for the spray of MSMA herbicide on rice with almost complete interception when the rice plants approached maturity. While a larger canopy cover can reduce the load of spray pesticide into the water compartment, the application of tricyclazole only depends on the occurrence of blast disease. Therefore, when preventative application is performed to rice in the early growth stage with low canopy cover, the water holding practice should be paid great attention to prevent the discharge of water with high concentration of tricyclazole to the environment.

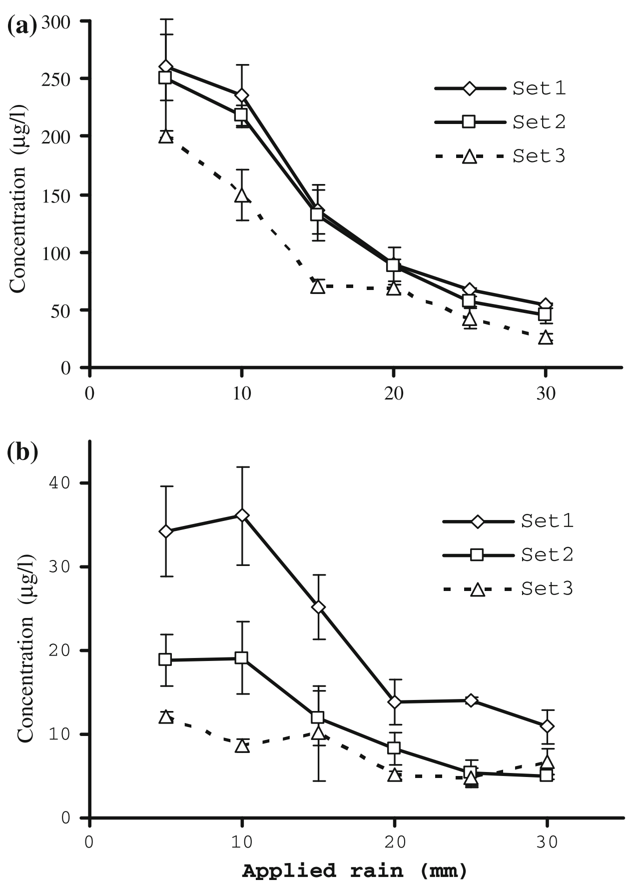

Fig. 1 Concentrations of tricyclazole in wash-off water in the first (**a**) and second (**b**) rainfall simulation

In both rainfall events, the concentration of tricyclazole washed from the rice leaves followed sigmoid curves (Fig. 1). The sigmoid shape of these curves is different from the popular hyperbolic shape of rapid foliar wash-off of herbicides and insecticides from grass, cotton and apple seedlings as reported in other studies (Reddy et al. 1994; Willis et al. 1994; Hunsch et al. 2007). It showed that the removal of tricyclazole from rice leaves was slower but steadier than in other studies as also discussed in later section. It is suggested that the wetting and desorption process in rice leaf was slower than in other plant species due to leaf surface characteristics, although the availability of tricyclazole on rice leaf before the first rainfall was relatively high. Yu et al. (2001) reported that about 90% of the applied tricyclazole could be extracted from leaf surface by a solvent mixture, 24 h after application. This extractable ratio was high compared to less than 70% of edifenphos and ~60% of isoprothiolane. However, the portion that can be extracted by pure water remains questionable. Reddy et al. (1996) has already mentioned the effect of leaf surface characteristics to the retention of imazaquin spray in two weed species but gave no detailed discussion. Therefore, detailed investigation is required to clarify the mechanism of the pesticide wash-off from rice leaf.

In the first rainfall, concentration of tricyclazole in wash-off water of set 1 was slightly higher than that of set 2 while wash-off concentration in set 3 was significantly lower than those in two other sets (Fig. 1a). Probably the application rate, the interval between spray time and rainfall simulation as well as the loss of tricyclazole through spray drift all contributed to the difference among sets. However, the main factor causing the significant deviation in set 3 was unclear. Wash-off concentrations in this rainfall event were high with maximum value of up to 288.2, 294.1 and 203.3 $\mu g\ L^{-1}$ in set 1, 2 and 3, respectively, after 5 mm of rainfall. Wash-off of tricyclazole shortly after its application may reduce the fungicidal effectiveness. Also, it may pose a risk to the environment because runoff of water containing pesticide can easily occur in the paddy field during rainfall events larger than 1.5 cm (Vu et al. 2006).

Figure 1b showed that tricyclazole deposit was still washed from leaf surface in the second rainfall, though only residual amount of tricyclazole was left from the first simulation. However, there were clear differences among experimental sets. Probably the difference in residual tricyclazole amount on leaf surface, due to different application rate, resulted in different wash-off concentrations. Maximum concentrations of tricyclazole in the second simulation were 34.3, 18.8 and 12.2 μg L^{-1} for set 1, 2 and 3, respectively.

Losses of tricyclazole by wash-off from rice foliage were calculated as percentage of the initial deposition mass. Data of tricyclazole cumulative losses as functions of cumulative rainfall applied to each set was presented in Fig. 2. Although the loss rate was higher in the earlier period of the simulation, the steepness of the slope was not changed significantly during the experiment. This behavior is different from the behavior of other pesticides reported in the literature where most of the wash-off loss occurred early in the wash-off event (Willis et al. 1992, 1994; Hunsch et al. 2007). In this study, 50% of the loss was incurred by 10 mm of rain while only about 3 mm of rain was needed to produce similar loss of mancozeb from apple seedling (Hunsch et al. 2007) or of azinphosmethyl from cotton plants (Willis et al. 1994). It should be noted that both mancozeb (water solubility: 5 mg L^{-1}) and azinphosmethyl (water solubility: 33 mg L^{-1}) are much more hydrophobic than tricyclazole. However, the eventual decrease of the slope steepness suggested a decline in the fraction of easily washed tricyclazole from the leaves with increasing time or rainfall depth.

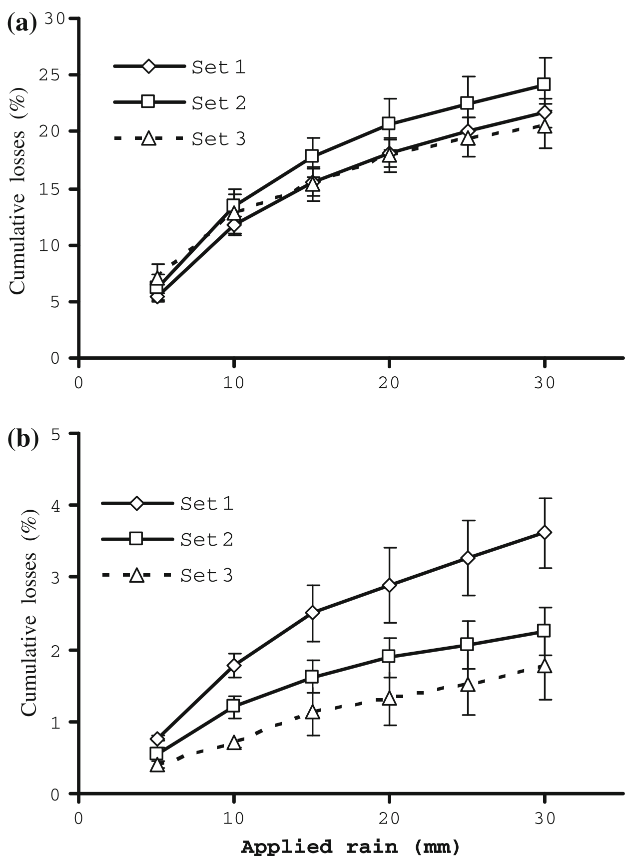

Fig. 2 Cumulative losses of tricyclazole through wash-off water in the first (**a**) and second (**b**) rainfall simulation

In the first simulation, there was not significant difference in losses among experiment sets as the consequence of similar wash-off concentration (Fig. 2a). Cumulative losses were 21.7%, 24.2% and 20.5% of tricyclazole initially deposited on rice leaves for set 1, 2, and 3, respectively. These losses corresponded to 14.1%, 17.9% and 15.7% of applied tricyclazole in set 1, 2, and 3, respectively. Those values were not significantly different although there were differences in drying time. Therefore, it is suggested that the rainfastness of tricyclazole formulation was independent from the interval between spray time and rainfall event. Similar results were also reported for mancozeb sprayed on apple seedling (Hunsche et al. 2007). However, many other studies observed the dependence of wash-off amount on the drying time of pesticide deposit (Willis et al. 1992, 1994). Therefore, each compound or formulation will need separate experiment in order to determine this property (Hunsche et al. 2007).

The extent of loss in this study was low compared to other studies. Less than 25% of the tricyclazole mass was washed from the rice foliage in a normal rainfall, although tricyclazole is relatively water soluble. Meanwhile, Hunsche et al. (2007) reported losses of up to 90% of applied mancozeb through wash-off from apple seedling and Reddy et al. (1994) reported that 51% and 82% of lactofen was removed from velvetleaf and cocklebur foliage, respectively. Wauchope et al. (2004) reviewed that many insoluble pesticides were relatively washable with losses ranging from 50% to 80%. Reason for this phenomenon is the presence of formulation components, which promote suspension and solubilization. It should be noted that the time interval practiced in the above mentioned studies was mostly less than 24 h after spray. However, our cases considered more realistic time interval. Therefore, the data obtained in this study can be useful for modeling pesticide behavior in rice plant.

There were significant differences in losses of the second simulation among sets (Fig. 2b). However, the extent of losses was considerably lower than those of the first simulation with the values of only 3.6%, 2.2% and 1.8% in set 1, 2 and 3, respectively. Besides the losses of tricyclazole during the first simulation the degradation of tricyclazole on the surface of the leaves may also help to reduce the amount washed by rainfall water in the second simulation.

The application of two consecutive normal rainfall simulations has shown that tricyclazole can be removed from rice leaves but in lesser extent than other pesticides. And most of the loss of foliar-applied tricyclazole occurred in the first rainfall event of just 30 mm. Extent of loss was not dependent on the interval between spray time and rainfall event. Although tricyclazole residue in leaf surface was still available for further wash-off in the second rainfall simulation of another 30 mm, the wash-off concentration was much lower than those in the first simulation and the loss by wash-off was not significant.

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