

## Methods of Applying Tricyclazole for Control of *Pyricularia oryzae* on Rice

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### ABSTRACT

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Tricyclazole [5-methyl-1,2,3-triazolo(3,4-b)benzothiazole] was evaluated for control of *Pyricularia oryzae* on rice by the following methods of application: soil drench to the seedling flat one day before mechanical transplanting, foliar sprays at different stages of growth, and combinations of soil drench plus foliar spray. Results from 42 field trials were summarized and compared with the performance of several reference fungicides applied according to manufacturers' specifications. Tricyclazole applied as a soil drench at 2.25 g/flat before transplanting effectively controlled leaf blast throughout most of the vegetative stage of growth and reduced rotten neck incidence by 50%. The most effective foliar spray application for rotten neck and panicle blast

control was 225-375 g/ha tricyclazole applied less than 10 days before heading (late booting stage). Earlier spray applications necessitated higher rates for equivalent disease control. When tricyclazole was sprayed on plants that had received a soil drench application before transplanting, 150 g/ha at late booting provided acceptable control of rotten neck. Tricyclazole rates for soil drench and foliar applications, as well as numbers and timing of foliar applications, must be determined for different rice-growing areas because of differences in local disease occurrence. Nevertheless, the proper use of tricyclazole should result in equivalent or superior rice blast control with fewer applications than with the fungicides currently being used.

Tricyclazole [5-methyl-1,2,4-triazolo(3,4-b)benzothiazole] is being developed as a new fungicide for control of *Pyricularia oryzae* Cav. on rice in Japan, Taiwan, and other countries where rice blast is a problem.

Although many fungicides have been introduced for disease control on rice, rice blast is still a major disease of that crop (2). Most fungicides registered in Japan require multiple foliar applications for full-season disease control. Some of the more-recently developed fungicides are efficacious when applied to the paddy water surface (2, 8). One compound has limited activity when applied to the soil surface of seedling flats before mechanical transplanting (7).

Tricyclazole controls *P. oryzae* on rice in field evaluations (3, 4, 5, 9). Results from early greenhouse and field studies show that tricyclazole is readily absorbed by roots, translocates to leaves, and provides residual disease control after a single soil or foliar application (1).

Studies were conducted in 1974 and 1975 to evaluate these properties of tricyclazole for rice blast control in the field. Application methods evaluated were: soil drench to the seedling flat prior to mechanical transplanting, foliar spraying at various growth stages, and combinations of both methods. The results summarized here are from 42 tests conducted by us and cooperating researchers in Japan.

### MATERIALS AND METHODS

A 75% wettable powder (WP) formulation of tricyclazole was used in all studies. Rates of application are stated in terms of grams (g) of active ingredient per hectare (ha) or per transplant flat.

Field studies were conducted in several locations in Japan using different rice cultivars of the japonica type. Correlations were not made regarding cultivar susceptibility to rice blast and the efficacy of tricyclazole. Plot sizes ranged from 10 to 100 m<sup>2</sup>.

Soil drench applications of tricyclazole were made to 2- to 3-wk-old seedlings growing in commercially available flats of standard size (28 × 58 × 3 cm). Tricyclazole was suspended in 0.5 liter water and poured uniformly over the soil in a flat approximately 24 hr before the seedlings were to be transplanted by machine. Approximately 200 flats of rice were transplanted per hectare. Soils in the flats were of different textures. It was suspected that soil texture influenced efficacy and crop response to tricyclazole; however, no attempt was made to correlate compound performance with soil texture in these studies.

Foliar applications of tricyclazole were made with single and multi-nozzle hand-carried spray booms operated from knapsack sprayers. Spray volume was 1,000 to 1,500 liters per hectare depending on the stage of rice growth. Variables evaluated were tricyclazole rates and timing of application. Timing was determined in relation to the growth stages of the rice plants (Table 1).

The reference fungicides used were blasticidin-S

(Kaken Chemical Co., Ltd., Tokyo, Japan), edifenphos (Nikon Tokushu Noyaku Seizo K. K., Tokyo, Japan), kasugamycin (Hokko Chemical Industry Co., Ltd., Tokyo, Japan), and Rabcide (4, 5, 6, 7-tetrachlorophthalide, Kureha Chemical Industry Co., Ltd., Tokyo, Japan). All were foliar-applied at rates and intervals recommended by their manufacturers.

Infection of rice by *P. oryzae* results in different symptoms depending on the plant part affected. Disease symptoms reported in this paper include leaf blast (LB, lesions on leaves), rotten neck (RN, decayed culm tissue below the panicle), and panicle blast (PB, decayed axis of the panicle, particularly where greater than two-thirds of the panicle was affected). Although additional disease symptoms were recorded from field studies (lesser degrees of PB and node blast, which is decay of the first node below the panicle), the above were considered the most important in determining the fungicidal efficacy of tricyclazole. Leaf blast intensity was estimated in several locations per plot as the percent leaf area covered by disease lesions. Rotten neck and panicle blast incidence were calculated on a percentage basis by counting all diseased and healthy plants in 20 hills per plot. There were approximately 10-15 tillers per hill. An overall disease index (DI) was computed from these data using a formula commonly used in Japan:

$$DI = \frac{[(4 \times RN) + (3 \times PB > 2/3) + (2 \times PB > 1/3 < 2/3) + (1 \times PB < 1/3)]}{4 \times 100}$$

where  $PB > 2/3$  means panicle blast (%) affecting greater than two-thirds of the panicle,  $PB > 1/3 < 2/3$  means panicle blast (%) affecting between one-third and two-thirds of the panicle, and  $PB < 1/3$  means panicle blast (%) affecting less than one-third of the panicle. In this formula, greater emphasis is placed on RN and  $PB > 2/3$  in deriving DI because those symptoms are more detrimental to rice yield and grain quality. A DI value of less than 20 for a treatment is generally interpreted as acceptable blast control (Kushiro, *personal observation*).

All treatments were replicated three times. Data from common treatments in separate experiments were

averaged for presentation in the tables. Since every treatment was not evaluated in each experiment, the summary tables represent mean values from a range of observations. For example, in Table 1, data on the efficacy of the 2.25 g/flat rate of tricyclazole were collected from 10 experiments in which leaf blast was recorded and 13 experiments in which rotten neck and panicle blast were recorded. Results for the 3.75 g/flat rate were collected from the same plus two additional experiments. This lack of uniformity was unfortunate because it made statistical evaluations less accurate. However, this method of summarization simplified a review of the results.

## RESULTS

When tricyclazole was applied as a soil drench before transplanting, leaf blast was effectively controlled after transplanting (Table 2). Although data from the various experiments summarized in Table 2 were recorded at different times after transplanting, many field observations have substantiated leaf blast control for approximately 50 days after transplanting. Leaf blast control was improved only slightly with tricyclazole rates above 2.25 g/flat.

No appreciable differences were seen in results which could be attributed to plot size. Location of plots, either adjacent to or far from diseased nontreated rice, was a greater factor in producing variable results.

Soil drench applications provided up to 50% control of rotten neck and panicle blast symptoms which appeared between 70 and 100 days after transplanting. That percentage of control was less than the control provided by reference fungicides which were foliar-applied according to manufacturer's recommendations. Lack of late-season disease control also was reflected in the high DI values. Although soil drench of tricyclazole in the present experiments provided excellent leaf blast control, it did not provide acceptable full season disease control.

A single foliar application of tricyclazole at the booting stage of growth provided rotten neck and panicle blast control comparable to the most active reference fungicides which were applied two to four times in the reproductive stage (Table 3). Application of tricyclazole

TABLE 1. Identification and description of rice growth stages

Growth stage	Description	Walker scale
Transplanting	Plants 14-28 days old, two- to four-leaf stage	Stage 2
Tillering	New shoot and leaf formation	Stages 3.1-3.9
Panicle initiation (ear primordia)	Beginning of reproductive stage, panicle formation inside leaf sheath	Stage 4 (early)
Booting (ear sprouting)	Panicle moving up inside leaf sheath	Stage 4 (mid & late)
Early heading	Panicle emergence from sheath	Stages 5.1-5.5
Full heading	100% panicle emergence and flowering	Stage 5.9
Ripening	Milk stage through dead ripe	Stages 6.1-6.9

TABLE 2. Control of *Pyricularia oryzae* on rice from soil drench application of tricyclazole in the seedling flat approximately 24 hr before transplanting by machine<sup>a</sup>

Fungicide	Rate (g/flat)	Tests conducted (no.)	Leaf blast (%)	Rotten neck (%)	Panicle blast (%)	Disease index <sup>b</sup> (DI)
Tricyclazole	2.25	10-13	1.3	16	12	32
Tricyclazole	3.75	11-15	1.1	15	10	29
Tricyclazole	5.25	7-11	1.0	16	9	32
Blasticidin-S	FA <sup>c</sup>	3-4	...	5	11	20
Edifenphos	FA	4	1.0	6	3	12
Rabcide	FA	6-11	4.6	8	8	19
Control	0	11-16	8.4	32	15	50
LSD ( $P=0.05$ )			3.3	8.6	4.0	10.8

<sup>a</sup>Data are means from multiple tests, but not all treatments were in every test. Refer to Materials and Methods for explanation.

<sup>b</sup>Disease index calculated from rotten neck and panicle blast incidence by formula discussed in Materials and Methods.

<sup>c</sup>Reference fungicides were foliar applied (FA) at rates and intervals recommended by the manufacturers: blasticidin-S 20-30 g/ha, edifenphos and Rabcide 500-750 g/ha; generally two applications for leaf blast and two for rotten neck and panicle blast control.

TABLE 3. Control of *Pyricularia oryzae* on rice by a single foliar spray of tricyclazole applied at different stages of growth<sup>a</sup>

Fungicide	Rate (g/ha)	Tests conducted (no.)	Growth stage	Rotten neck (%)	Panicle blast (%)	Disease index <sup>b</sup> (DI)
Tricyclazole	525-1125	3	Tillering	12	13	19
Tricyclazole	525	5	Booting	4	2	9
Tricyclazole	525	1	Early heading	10	5	21
Tricyclazole	525	2	Full heading	15	6	30
Blasticidin-S <sup>c</sup>	20-30	1	4 Applications	5	4	16
Edifenphos	500-750	2	2-4 Applications	6	4	14
Kasugamycin	20-30	1	2 Applications	33	10	54
Rabcide	500-750	7	2-4 Applications	12	7	16
Control	0	8	...	36	9	48
LSD ( $P=0.05$ )				19.7	8.4	22.1

<sup>a</sup>Data are means from multiple tests, but not all treatments were in every test. Refer to Materials and Methods for explanation.

<sup>b</sup>Disease index is calculated from rotten neck and panicle blast incidence by the formula discussed in Materials and Methods.

<sup>c</sup>Reference fungicides were foliar applied two to four times as recommended by the manufacturers.

TABLE 4. Control of *Pyricularia oryzae* on rice from a single foliar spray of tricyclazole applied at different days before heading (DBH)

Fungicide	Rate (g/ha)	Application greater than 10 DBH				Application less than 10 DBH			
		Tests conducted (no.)	Rotten neck (%)	Panicle blast (%)	Disease index <sup>a</sup> (DI)	Tests conducted (no.)	Rotten neck (%)	Panicle blast (%)	Disease index <sup>a</sup> (DI)
Tricyclazole	150	3	20	14	58	8	11	4	19
Tricyclazole	225	3	23	14	58	8	10	3	18
Tricyclazole	375	3	25	15	52	6	8	3	16
Tricyclazole	480	1	10	26	27	1	3	4	14
Tricyclazole	750	4	2	2	8	4	0.5	1	4
Rabcide <sup>b</sup>	500-750	8	3	14	11	13	5	3	14
Control	0	8	28	11	44	13	23	11	37
LSD ( $P=0.05$ )		rotten neck		12.2%		panicle blast		3.5%	
		disease index		12.8 DI units					

<sup>a</sup>Disease Index calculated from rotten neck and panicle blast incidence by formula discussed in Materials and Methods.

<sup>b</sup>Reference fungicide was foliar applied two to four times as recommended by the manufacturer.

at tillering or heading did not provide acceptable rotten neck control. Heading stage applications did provide marginally acceptable panicle blast control.

The booting stage can encompass a time period of up to 21 days depending on cultivar and weather. To determine whether early or late booting applications of tricyclazole differed in efficacy, data from 1975 experiments were separated according to time of application: greater than 10 days before heading (DBH) and less than 10 DBH. Results shown in Table 4 clearly illustrate that tricyclazole was more effective in reducing rotten neck and panicle blast incidence when applied less than 10 DBH. This time factor could be overcome by increasing the tricyclazole rate. For example, 480 g/ha applied greater than 10 DBH provided rotten neck control equivalent to 225 g/ha applied less than 10 DBH. Furthermore, to provide disease control similar to multiple applications of the reference fungicide, it required 750 g/ha applied greater than 10 DBH but only 375-480 g/ha applied less than 10 DBH.

Tricyclazole was next evaluated for full season blast control from a combination soil drench application before transplanting followed by a foliar spray at less than 10 DBH (Table 5). The soil drench application again provided excellent leaf blast control at 2.25 g/flat. Control of rotten neck and panicle blast by tricyclazole was not significantly different from control provided by multiple applications of reference fungicides. Disease control improved slightly with increased rates of tricyclazole. However, acceptable control was achieved in these experiments from the lowest rates in the combination (2.25 g/flat + 150 g/ha).

Rotten neck control from the combination of tricyclazole treatments was better than from the foliar spray of tricyclazole alone. (Refer to 150 g/ha rate in

Tables 4 and 5). This result indicates that the soil drench application before transplanting contributed to rotten neck control. (Refer to Table 2 for supporting evidence.) Thus, lower tricyclazole rates were effective at booting for rotten neck control if a soil drench of tricyclazole was applied before transplanting.

A soil drench of tricyclazole occasionally was phytotoxic to seedlings. The injury appeared within 1 wk after transplanting, and only on leaves present at time of treatment. The severity of injury was greater at high application rates (5.25 g/flat) than at low rates (2.25 g/flat). Injury symptoms ranged from chlorotic to necrotic leaf tips. New leaves appeared normal. Subsequent measurements of plant height, number of tillers, and yield showed no adverse effects from tricyclazole treatments. No phytotoxicity ever has been associated with foliar sprays of tricyclazole at rates up to 2 kg/ha.

## DISCUSSION

Tricyclazole is a highly efficacious fungicide for control of *P. oryzae* on rice. An earlier report (1) described the different methods by which tricyclazole could be applied to provide blast control, its systemic movement in the plants, and residual effectiveness. Field performance of tricyclazole is summarized in the present report and in the report by Yamaguchi (9).

A soil drench of tricyclazole to seedlings at 2.25 g/flat 1 day before mechanical transplanting protected rice from leaf infection as effectively as multiple foliar applications of currently used fungicides. Current fungicides are effective if applied frequently and at proper times in relation to infection periods. However, often the applications are not timed properly to effect optimum leaf

TABLE 5. Control of *Pyricularia oryzae* on rice by tricyclazole applied as a soil drench (SD) application to the seedling flat approximately 24 hr before mechanical transplanting followed by a single foliar application (FA) less than 10 days before heading<sup>a</sup>

Fungicide	Applications		Tests conducted (no.)	Leaf blast (%)	Rotten neck (%)	Panicle blast (%)	Disease index <sup>b</sup> (DI)
	SD (g/flat)	FA (g/ha)					
Tricyclazole	2.25	150	8-13	1.4	6	8	18
Tricyclazole	2.25	225	8-12		5	7	18
Tricyclazole	2.25	375	7-11		6	6	19
Tricyclazole	3.75	150	10-18	0.8	4	5	14
Tricyclazole	3.75	225	12-19		4	5	14
Tricyclazole	3.75	375	10-17		3	4	12
Tricyclazole	5.25	150	5-7	1.0	4	6	17
Tricyclazole	5.25	225	5-7		2	6	14
Tricyclazole	5.25	375	6-8		3	5	12
Blasticidin-S <sup>c</sup>	0	20-30	1-2	...	6	7	14
Edifenphos	0	500-750	4-5	1.0	6	6	17
Rabcide	0	500-750	11-20	2.8	6	6	14
Control	0	0	14-25	5.3	31	14	47
LSD ( $P=0.05$ )				2.2	6.9	3.8	7.1

<sup>a</sup>Data are means from multiple tests, but not all treatments were in every test. Refer to Materials and Methods for explanation.

<sup>b</sup>Disease index is calculated from rotten neck and panicle blast incidence by the formula discussed in Materials and Methods.

<sup>c</sup>Reference fungicides were foliar applied two to four times as recommended by the manufacturers.

blast control. Application of tricyclazole before transplanting protects rice from leaf blast for approximately 50 days regardless of the number and frequency of infection periods.

Proper timing of a single foliar spray is critical for maximum rotten neck and panicle blast control from low rates of tricyclazole. If the application is not made within 10 DBH, higher rates may be required. Under unusually severe disease conditions, where weather favorable for disease extends from late booting to harvest, a second tricyclazole application may be necessary. However, two foliar applications of tricyclazole under these conditions are still fewer than the number required of currently used fungicides.

Outstanding full-season rice blast control has been obtained from the combination soil drench and foliar spray applications of tricyclazole. Results from the experiments summarized in this report indicate that the lowest rates evaluated during 1975 (2.25 g/flat + 150 g/ha) provide acceptable disease control. Higher rates improve disease control; however, the differences are not statistically significant. Since time and severity of disease occurrence differ in various areas of rice cultivation, different rates and times of tricyclazole application no doubt will be required to provide acceptable disease control. Nevertheless, a tricyclazole program for blast control should reduce frequency of fungicide application and improved disease control.

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