**MBI-D resistance management of *Pyricularia oryzae* using an application program incorporating benomyl**

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This study’s objective was to evaluate the contributions of benomyl and a melanin biosynthesis dehydratase inhibiotor (MBI-D) mixture to preventing the re-emergence of MBI-D-resistant *Pyricularia oryzae* in rice fields. In nursery boxes, applications of diclocymet or mixtures of diclocymet and tiadinil showed low leaf blast incidences, but MBI-D-resistant isolates were re-selected. However, a management program applying both benomyl and MBI-D-related products to nursery box plants prevented the re-emergence of MBI-D-resistant isolates more effectively than the application of only MBI-D-related products. © Pesticide Science Society of Japan

Keywords: resistance management, benomyl, diclocymet, rice blast.

Rice blast caused by *Pyricularia oryzae* is the most serious rice disease in East Asia, especially Japan. The melanin biosynthesis pathway is important for *P. oryzae*'s capability to infect rice. At present, there are two known groups of melanin biosynthesis inhibitors that control rice blast. One is a group of 1,3,6,8-tetra- and 1,3,8-trihydroxynaphthalene reductase inhibitors of melanin biosynthesis (melanin biosynthesis reductase inhibitors, MBI-Rs), such as tricyclazole and pyroquilon. The other is a group of scytalone dehydratase (SH) inhibitors of melanin biosynthesis (melanin biosynthesis dehydratase inhibitors, MBI-Ds). This group of fungicides includes carpropamid, diclocymet, and fenoxanil, which were introduced in Japan in 1998, 2000, and 2001, respectively. Both carpropamid and diclocymet are mainly applied to nursery boxes to control rice blast in paddy rice fields. *P. oryzae* is classified as a pathogen that has a high risk of developing resistance to fungicides as assessed by the Fungicide Resistance Action Committee. Isolates resistant to carpropamid were first reported in 2001 in Saga Prefecture, and these isolates showed cross-resistances to other MBI-D fungicides. A single-point mutation in the SH gene (GenBank Accession Number AB004741), which causes the substitution of one amino acid in SH (valine 75 to methionine: V75M; R-mutation), was found in the carpropamid-resistant isolates. Suzuki et al. monitored the emergence frequency of resistant isolates in fields in Kyushu after the cessation of MBI-D applications and showed that the frequency declined. Kimura and Fujimoto reported that the emergence frequency of resistant isolates decreased rapidly without MBI-D's pressure in the laboratory and field tests. These previous reports suggested that MBI-D-resistant isolates paid a fitness penalty for acquiring their resistance and that sensitive isolates would be dominant without MBI-D pressure. However, the re-use of MBI-Ds has not been implemented in the area where resistant isolates were widely found. In general, an application program using several fungicides whose modes of action are different, or using a mixture of single-site fungicides with multisite fungicides, is recommended as the management strategy for fungicide resistance. However, MBI-D resistance was the first case of resistance to fungicides applied to nursery boxes worldwide, and at present, management strategies for MBI-D resistance have not been thoroughly investigated. Thus, there are no sound resistance management practices to prevent the re-emergence of MBI-D-resistant isolates.

In this study, we searched for a resistance management practice by testing application programs using fungicides that have different modes of action. We focused on nursery box applications at sowing and planting times. Rice blast has a seed-borne disease dimension. Seeds are the most important carriers of *P. oryzae*. We hypothesized that an application that reduced the population of seed-borne rice blast was a key factor in resistance management practice. A previous study reported that benomyl controlled seed-borne rice blast. Thus, we incorporated benomyl into the fungicide application programs. Additionally, we used a mixture of diclocymet and tiadinil, which uses systemic acquired resistance as its mode of action, as a mixed fungicide. We evaluated the contributions of benomyl and the mixture in maintaining low frequencies of resistant-isolate emergence in a rice field.

One rice field in Ehime Prefecture in 2007 was used for the study. The frequency of MBI-D-resistant isolates in this area was almost 100% in 2003, and MBI-D applications were halted in 2004. Thereafter, the frequency of MBI-D-resistant isolates declined yearly, and by 2006, the frequency was almost 0%. The chemicals used in this study are shown in Table 1 and were purchased from an agricultural cooperative. At sowing time, 500 mL of 1,000 mg L⁻¹ benomyl was drenched into a nursery box. The other products were applied to the nursery box at
Table 1. Products used in these experiments

| Common name | Target pest | Content (%) | Formulation |
|-------------|-------------|-------------|-------------|
| benomyl     | blast       | 50          | wettable powder |
| diclocymet  | blast       | 1.5         | granule     |
| tiadinil    | blast       | 6           |             |
| furametpyr  | sheath blight | 4         |             |
| chloethianidin | insect     | 1.5         |             |
| diclocymet  | blast       | 3           | granule     |
| furametpyr  | sheath blight | 4         |             |
| fipronil    | insect      | 1           |             |
| probenazole | blast       | 24          | granule     |
| fipronil    | insect      | 1           |             |

Table 2. Frequency of the MBI-D-resistance mutation in Pyricularia oryzae per plot

| Applied product | No. of leaves | No. of R-mutations | R-mutations (%) |
|-----------------|---------------|--------------------|-----------------|
| Sowing          |               |                    |                 |
| benomyl         | 27            | 1                  | 3.7             |
| none            | 21            | 5                  | 23.8*            |
| benomyl         | 18            | 2                  | 11.1            |
| none            | 16            | 4                  | 25.0*            |
| benomyl         | 20            | 0                  | 0               |
| none            | 27            | 0                  | 0               |
| benomyl         | 28            | 2                  | 7.1             |
| none            | 25            | 1                  | 4.0             |
| Planting        |               |                    |                 |

*Application dates: May 13 (sowing) and June 13 (planting). *The mutation V75M in scytalone dehydratase (SH) was analyzed using primer-introduced restriction enzyme analysis PCR. *Statistically different from the reference plot (none-probenazole) according to Fisher’s exact test (p = 0.05).
MBI-D’s resistance management by benomyl

Performance in controlling rice leaf blast. However, none-diclocymet and none-diclocymet+tiadinil programs predictably re-selected R-mutations more than the none-probenazole program did. Thus, successive applications of MBI-D, year after year, without the inclusion of other active ingredients with different modes of action, may lead to the re-selection of resistant isolates. Thus, it is important to establish sound resistance management strategies for areas where sensitive isolates are likely to become dominant. Benomyl–diclocymet and benomyl–diclocymet+tiadinil programs not only resulted in high rice leaf blast control but also maintained lower frequencies of the R-mutation than the none-MBI-D-related product programs. Because these results were from one field trial, it is necessary to conduct more field trials to confirm that the benomyl-incorporated program is efficient in preventing the re-emergence of MBI-D-resistant isolates. We hypothesized that benomyl reduced the total number of resistant conidia in the nursery box during the early rice season and thus prevented the re-emergence of resistant isolates by MBI-D-related products. Further fungicide studies are necessary to prove our hypothesis. In this study, we used benomyl to control seed-borne rice blast. However, some other fungicides are registered to control seed-borne rice blast in Japan. Thus, further studies are necessary to evaluate the effectiveness of other fungicides to maintain a low frequency of the R-mutation. We believe that incorporating benomyl into a program using MBI-D-related products is a possible strategy for the re-use of MBI-D products in fields where MBI-D performance has recovered. This is the first report to indicate the contribution of benomyl to MBI-D resistance management. Recently, QoI-resistant P. oryzae isolates were detected in many prefectures in Japan. This study provides important knowledge for the management of resistance to other fungicides, such as QoI.

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Table 3. Disease incidence of leaf blast in each plot

| Applied product | Incidence (%) |
|-----------------|--------------|
|                 | Sowing\(a\) | Planting\(a\) |
| benomyl         |            |              |
| diclocymet+tiadinil | 22.2** |              |
| none            |            |              |
| diclocymet+tiadinil | 24.7** |              |
| benomyl         |            |              |
| diclocymet      | 28.0**     |              |
| none            |            |              |
| diclocymet      | 32.0**     |              |
| benomyl         |            |              |
| probenazole     | 43.0*      |              |
| none            |            |              |
| probenazole     | 44.3*      |              |
| benomyl         |            |              |
| none            |            |              |
| none            | 99.7#      |              |

\(a\) Application dates: May 13 (sowing) and June 13 (planting).
\(b\) Percentage of diseased plants among 300 plants. *Statistically different from the untreated (none–none) plot based on the \(\chi^2\) test (\(p=0.05\)). **Statistically different from the reference (none-probenazole) plot based on the \(\chi^2\) test (\(p=0.05\)).