Fusarium graminearumに対する新規キレート殺菌剤カルベンダジム-8-オキシキノリン-の効果

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Original Article

Effect of carbendazim-8-oxyquinoline-copper, a novel chelate fungicide against *Fusarium graminearum*

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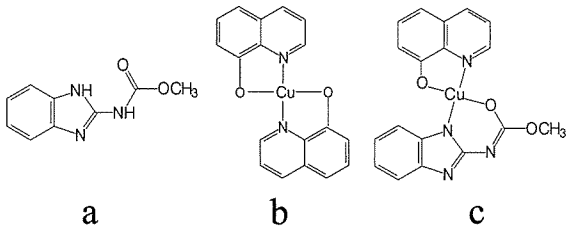
The efficacy of carbendazim-8-oxyquinoline-copper (BCOC), which is a chelate of carbendazim (MBC) and oxine-copper (BHC), was compared with that of MBC and BHC for control of *Fusarium graminearum*. *In vitro* inhibition of MBC-resistant (MBC^R) strains was greater with BCOC than with MBC or BHC, or the mixture of MBC and BHC; the minimum inhibitory concentration (MIC) of MBC-high-resistant strains for BCOC was 10 times lower than the MIC for MBC. Appearance of germ tubes of *F. graminearum* was similar after treating with BCOC or MBC. The EC_{50} of BCOC was 1.29±0.61 μg/mL against 124 *F. graminearum* field strains collected in 2010 and 3.23±0.41 μg/mL against 127 *F. graminearum* MBC^R field strains collected between 2000 and 2010. BCOC provided best protective and curative control of Fusarium head blight on wheat artificially inoculated with both MBC^S (MBC sensitive) and MBC^R strains in the field. To explain the high efficacy of BCOC, we hypothesize that the 8-oxyquinoline-copper in BCOC changes the molecule's structure and thereby increases the interaction between the carbendazim radical in BCOC and the β_2-tubulin protein in *F. graminearum*. BCOC has substantial potential for the synthesis of new fungicides for disease control and resistance management.

Keywords: carbendazim-8-oxyquinoline-copper, carbendazim, oxine-copper, *Fusarium graminearum*, resistant, susceptibility.

Introduction

Fusarium head blight (FHB) results in the contamination of wheat and other cereal crops with mycotoxins such as deoxynivalenol (DON), nivalenol (NIV), T-2 toxin, zearalenone (ZEA), and their derivatives; these mycotoxins threaten food safety and are a global concern.[1,2] In China, where FHB is mainly caused by *Fusarium graminearum*, control of FHB for over 30 years has largely depended on the application of carbendazim (methyl benzimidazole-2-yl carbamate or MBC, Fig. 1a).[3,4] The continued use of MBC, however, is threatened by the development of MBC resistance in *F. graminearum* populations.[5,6] Previous studies demonstrated that the resistance was based on site-directed mutations in the β_2-tubulin gene.[7] Alternatives to MBC with novel modes of action are therefore needed to control FHB.

One alternative to MBC is carbendazim-8-oxyquinoline-copper (benzimidazole-2-carbamate-8-oxyquinoline-copper or BCOC, Fig. 1c), which is a chelate of MBC and oxine-copper (bis-(8-hydroxyquinolinato)-copper II or BHC, Fig. 1b). BHC is the most active of the metal oxinates and has been used as an agricultural fungicide for many years.[8–10] BHC is



a b c

Fig. 1. Chemical structures of (a) MBC, (b) BHC, and (c) BCOC.

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more effective, more durable (*i.e.*, less likely to select for resistance), and less toxic to nontarget organisms than many other copper compounds but has been used infrequently.[11]

The objectives of this research were to: (1) compare the *in vitro* sensitivity of *F. graminearum* to BCOC, BHC, and MBC; (2) compare the efficacy of BCOC, BHC, and MBC for control of FHB in the field, and (3) investigate the fungicidal mechanism of BCOC.

Materials and Methods

1. *Fungicides and media*

BCOC as a nonformulated compound (98.0% pure) and as a 50.0% wettable powder (WP) was provided by Shanghai Bright Green Chemical Industry Co. Ltd., China. MBC as an active compound (98.0%) and as a 50.0% WP was provided by Shenyang Chemical Industry Academy, China. BHC as an active compound (99.0%) and as a 33.5% suspension concentrate (SC) was provided by Zhejiang Haizheng Chemical Industry Co. Ltd., China.

For *in vitro* experiments, nonformulated BCOC and MBC were dissolved in 0.1 M HCl whereas nonformulated BHC was passed through a 600-mesh sieve (pore diameter 23 μm) and then suspended in a mixture of methanol and deionized water (1 : 1, v : v); the BHC suspension was shaken vigorously before use. All stock solutions were prepared at 10,000 μg/mL and stored at 4°C in the dark to preserve fungicidal activity. For testing *in vitro* inhibition of mycelial growth and conidium germination, the fungicides were serially diluted in sterile potato dextrose agar (PDA) at about 50°C, which was made from 200 g potato extract, 15 g agar, and 20 g dextrose per liter of H_2O.[4,7]

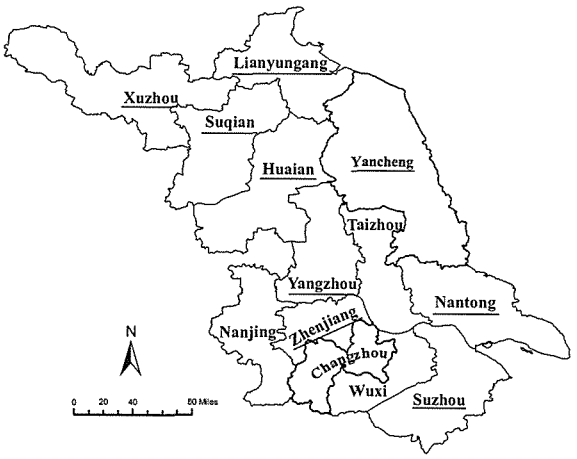

Fig. 2. Map of Jiangsu Province of P. R. China. Areas where *F. graminearum* was collected in May 2010 are underlined.

2. *Origin of* F. graminearum *isolates*

Of the 251 *F. graminearum* isolates used for determination of baseline sensitivitiy to BCOC (see section 2.4), 124 were randomly selected from 895 isolates collected from commercial wheat fields in Jiangsu Province, China in May 2010. These wheat fields were located at least 10 km from each other and in areas where BCOC had never been used (Fig. 2). Single-macroconidia isolates of *F. graminearum* were obtained using standard procedures as described by Chen *et al.*[12] The numbers of isolates obtained from each city of Jiangsu Province were: Zhenjiang (5), Suzhou (23), Nantong (17), Suqian (12), Huaian (25), Yangzhou (11), Yancheng (21), Lianyungang (6), Xuzhou (4). The other 127 isolates were collected from

Table 1. Effect of MBC, BCOC, and BHC (as indicated by EC_{50} and MIC values) on the *in vitro* mycelial growth of *F. graminearum* strains that differed in MBC susceptibility

Strain	Mutation site and phenotype to MBC[a]	MBC		BCOC		BHC	
		EC_{50}	MIC[b]	EC_{50}	MIC	EC_{50}	MIC
JT04	198, HR	5.6 bc[c]	>200	3.8 a	20	14.9 bc	>200
ZF43-17	198, IHR	1.3 cd	200	2.4 ab	20	13.9 bc	>200
NT-7	200, MR	7.2 b	50	2.2 ab	20	17.2 bc	>200
T1	200, MR	7.3 b	50	2.3 ab	20	11.3 c	200
R9	167, MR	6.6 b	70	2.3 ab	20	9.6 c	>200
ZF52	167, MR	9.5 b	50	2.4 ab	10	14.3 bc	>200
ZF52-7	167+17, IHR	43.4 a	>200	2.9 ab	25	24.3 b	>200
2021	wild-type, S	0.6 d	1.4	1.1 b	2.5	20.0 bc	>200
DB6	β_1-tubulin knockout, S	0.8 d	1.4	1.3 b	2.5	26.2 b	>200
DN83	β_2-tubulin knockout, S	0.1 d	0.4	0.2 b	0.6	41.0 a	>200

[a] HR: highly resistant; IHR: induced highly resistant; MR: moderately resistant; S: sensitive; JT04, NT-7, T1, R9, ZF52, and 2021 were wild-type strains; ZF43-17, ZF52-7, DB6, and DN83 were artificially modified strains. [b] Units for EC_{50} and MIC are μg/mL. [c] Values in a column followed by the same letter are not significantly different according to Fisher's LSD test (p=0.05).

various provinces in China from 2001 to 2010 and were determined to be MBC resistant (data not shown).

In addition to the 251 strains referred to in the previous paragraph, 10 other strains of *F. graminearum* were collected from different provinces of China from 2000 to 2010 and have been maintained in our laboratory. Seven were MBC-resistant (MBC^R)[3,7] and three were sensitive (MBC^S)[3,7,13] (Table 1). Some of these strains were artificially modified by induction (data not shown).

3. *Mycelial growth of* F. graminearum in vitro *as affected by BCOC, MBC, and BHC*

The 10 strains listed in Table 1 were used for this experiment. A 5-mm diameter mycelial disk cut from the edge of an actively growing culture (3 days old) of each strain was placed in the center of a 9-cm-diameter Petri dish containing PDA plus fungicide. The concentrations of MBC were 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.3 and 1.4 μg/mL for the MBC^S strains 2021 and DB6; 0.01, 0.02, 0.04, 0.08, 0.12, 0.16, 0.2, 0.3 and 0.4 μg/mL for the MBC^S strain DN83; and 1.56, 3.13, 6.25, 12.5, 25, 50, 60, 70, 80, 90, 100, 150 and 200 μg/mL for the MBC^R strains. The concentrations of BCOC were 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0, 2.1, 2.2, 2.3, 2.4, and 2.5 μg/mL for strains 2021 and DB6; 0.01, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.4, 0.5, and 0.6 μg/mL for strain DN83; and 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20 and 25 μg/mL for the MBC^R strains. The concentrations of BHC were 2.5, 5, 10, 20, 30, 40, 50, 100, and 200 μg/mL for all 10 *F. graminearum* strains. Control treatments contained PDA without fungicide and the experiment was performed twice with three replicates. After 3 days at 25°C, colony diameter of each strain was measured with the original mycelial disk diameter (5 mm) subtracted from this measurement. Percentage inhibition was calculated as $(1-a/b)\times 100$, where a is the colony diameter in Petri dishes with fungicide and b is the mean colony diameter in Petri dishes without fungicide. The median effective concentration (EC_{50}) values was calculated, and the minimum inhibitory concentration (MIC), *i.e.*, the minimum concentration that completely inhibited mycelial growth, was determined as the concentration at which no mycelial growth was observed.[3,7]

A mixture of MBC and BHC (referred to as M&B) was also used in this experiment. The molecular ratio of MBC to BHC was 2 : 1, which is the same ratio of quinoline to carbendazim in BCOC. M&B was tested at 10 and 50 μg/mL. The effect of M&B against the seven MBC^R strains listed in Table 1 (with three replicate plates per treatment) was compared with that of BCOC at the same concentrations.

4. *Germination of* F. graminearum in vitro *as affected by BCOC, MBC, and BHC*

Strain 2021 (sensitive to MBC, MBC^S, Table 1) and JT04 (highly resistant to MBC, MBC^{HR}, Table 1) were grown in 150-mL flasks containing 3% mung bean filtrate (filtrate of 30 g mung bean in 1 L H_2O). The flasks with medium, which were inoculated with 10 mycelial disks, were shaken at 170 rpm at 25°C and with a 12-hr photoperiod. After 5 days, conidia were collected and rinsed, and their concentration was adjusted to 1×10^5 CFU/ml with sterile water. A 20-μL volume of the conidial suspension was spread on a 20×20 mm coverglass, which was placed on cellophane on the surface of PDA plates with or without (control) fungicide; before placement on the cellophane, the coverslips were inverted so that the side with conidia was in contact with the cellophane. For determination of inhibitory effects, the concentration of BCOC and BHC in the agar was 1.5 and 5 μg/mL, respectively. For determination of completely inhibitive effects, the concentrations of BCOC and BHC in the agar were 5 and 10 μg/mL, respectively. Because a high concentration of MBC caused no inhibition of conidium germination of JT04, MBC was only used at 100 μg/mL, whereas the concentration for determination of the inhibitory and completely inhibitive effects was 1.0 and 5 μg/mL for strain 2021 respectively. There were three replicate coverslips for each treatment. After the coverslips with conidia had been on the agar for 8 hr at 25°C and without illumination, the conidia and germlings were photographed at 10×40 magnification with an OLYMPUS IX71 microscope equipped with an OLYMPUS DP72 lens. Image-Por Express version 6.0.0.319 (Media Cybernetics Inc.) was used for acquisition of photomicrographs at 4140×3096 pixels.

5. *Baseline EC_{50} values of BCOC against* F. graminearum

Baseline EC_{50} values (*i.e.*, EC_{50} values for *F. graminearum* populations that had never been exposed to BCOC) were determined for the 124 isolates of *F. graminearum* isolates collected from Jiangsu Province in May 2010, as described by Wang *et al.*[14] EC_{50} values were determined by measuring mycelial growth in agar containing BCOC at 0, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, and 6.4 μg/mL, as described in section 2.3.

Baseline EC_{50} values of BCOC were also determined for the other 127 *F. graminearum* isolates that were collected from different provinces of China between 2000 and 2010 and that had been determined to be resistant to MBC (MIC>2 μg/mL, unpublished data). BCOC concentrations of these resistant isolates were 0, 1, 2, 3, 4, 5, 6, and 7 μg/mL.

6. *Efficacy of BCOC* in vivo

F. graminearum strains 2021 (MBC^S) and JT04 (MBC^{HR}) and *F. graminearum*-susceptible wheat cultivar Yangmai 158 were used to compare the efficacy of BCOC (50% WP), MBC (50% WP), and BHC (33.5% SC) against FHB *in vivo*. The experiment was conducted at a field site (7.9×4.4 m) planted with wheat at the Pailou Experimental Centre of Nanjing Agricultural University. The wheat was planted on 30 October 2009 in small circular plots (0.2 m in diameter) with 10 plants per plot, and plots at 0.5 m intervals. A randomized complete block design was used. There were seven fungicide treatments: two rates (400 and 800 μg/mL) of each of the three

fungicides and a water control. Application was processed the day before and after artificial inoculation with each of the two strains, respectively. Overall, there were 28 treatments (7 combinations of fungicide and rate+water control×2 application times×2 strains of the fungus), and each treatment was represented by three replicate plots, giving 84 plots. On day 1 (28 April 2010), the plants in three replicate plots (42 of the 84) were sprayed with 50 ml fungicide or water; the wheat was flowering at this time. When the fungicide was applied, a plastic board was used to prevent the fungicide from contacting plants in adjacent plots, and all 10 plants in each plot were completely sprayed. On day 2, one mid-level spikelet on each plant in all 84 plots was injected with 10 μL conidial suspension (1×10^5 CFU/mL) of either strain 2021 or strain JT04. On day 3, plants that had not been sprayed on day 1 (plants in the remaining 42 plots) were sprayed with the same fungicides or with water. Fungicide application on day 1 (1 day before inoculation) was used to measure protective control while application on day 3 (1 day after inoculation) was used to measure curative control. The conditions in the field were suitable for FHB development (25–30°C and 85% RH), and no natural FHB developed in this field. FHB severity was rated 20 days after inoculation using the following scale: 0, no visible symptoms; 1, 1–3%; 2, 4–10%; 3, 11–25%; 4, 26–50%; 5, 51–75%; 6, >75% of the spikelets showed FHB symptoms.[15] The disease index=[n(1)+n(2)+n(3)+n(4)+n(5)+n(6)]/number of spikelets infected×100, where n indicates the number of infected plants rated as 1, 2, 3, 4, 5, or 6. Percentage disease control was calculated with the following formula: Percentage disease control=[1−(disease index of the treatment)/(disease index of water-treated control)]×100.

7. *Statistical analysis*

All data were subjected to analysis of variance (ANOVA). EC_{50} values were estimated after log-logit transformation of dose–response data. Means were separated with Fisher's least significant difference (LSD) at $p=0.05$. The software used was SAS GLM (SAS Institute Inc., Cary, NC).

Results

1. In vitro *sensitivity of* F. graminearum *mycelial growth to BCOC, BHC, and MBC*

There was no evident regularity of MBC^R strains for MBC and BCOC based on EC_{50} values, but the MIC values of MBC^{MR} and MBC^{HR} strains were obviously classified for MBC (Table 1); however, EC_{50} and MIC values of MBC^{MR} and MBC^{HR} strains were not significantly different for BCOC. BCOC was more inhibitive than MBC or BHC based on EC_{50} and MIC values except ZF43-17, the EC_{50} of which was slightly lower for MBC than for BCOC (Table 1), whereas the EC_{50} and MIC values of MBC^S strains were slightly higher for BCOC than for MBC. BHC was much less inhibitory than BCOC or MBC (Table 1).

Mycelial growth of all 7 MBC^R strains was strongly inhibited by 10 μg/mL BCOC and completely inhibited by 50 μg/mL BCOC, whereas 50 μg/mL M&B could hardly provide greater inhibition than 10 μg/mL BCOC. There was no significant difference in inhibition against strains treated with BCOC (Table 2).

2. *Germination of* F. graminearum in vitro *as affected by BCOC, MBC, and BHC*

Inhibition of conidium germination and germling growth of MBC^R strain JT04 was much greater with BCOC than with MBC (Fig. 3a–d), whereas inhibition of MBC^S strain 2021 was similar for the two fungicides (Fig. 3g–k). BCOC-treated and MBC-treated conidia that germinated produced multiple, distorted germ tubes and tended to germinate from the middle of the conidia (Fig. 3b, c, h, j). Although 10 μg/mL BHC could inhibit the germination of the two strains completely, it caused no change to the appearance of germ tubes (Fig. 3e, f, l, m). None of the three fungicides induced deformity of the

Table 2. Inhibition of mycelial growth of MBC-resistant *F. graminearum* strains by BCOC and M&B (MBC+BHC)

Strain	Mutation site and MBC phenotype[a)]	Percentage inhibition (means±SD)			
		BCOC at 10 μg/mL	M&B[b)] at 10 μg/mL	BCOC at 50 μg/mL	M&B at 50 μg/mL
JT04	198, HR	90.6±0.05 b[c)]	69.8±0.07 b	100±0.0 a	81.7±0.10 c
ZF43-17	198, IHR	90.3±0.07 b	82.1±0.10 a	100±0.0 a	94.7±0.02 a
ZF52-7	167+17, IHR	91.0±0.05 b	27.0±0.14 e	100±0.0 a	51.0±0.12 d
ZF52	167, MR	100±0.0 a	40.6±0.05 d	100±0.0 a	88.5±0.03 b
R9	167, MR	91.2±0.07 b	68.0±0.25 b	100±0.0 a	90.3±0.03 b
NT-7	200, MR	90.4±0.10 b	69.9±0.30 b	100±0.0 a	90.2±0.04 b
T1	200, MR	89.6±0.38 b	49.9±0.06 c	100±0.0 a	90.4±0.02 b

[a)] HR: highly resistant; IHR: induced highly resistant; MR: moderately resistant; JT04, ZF52, R9, NT-7 and T1 were wild-type strains; ZF43-17 and ZF52-7 were artificially modified strains. [b)] Molecular ratio of MBC to BHC=2 : 1. [c)] Values in a column followed by the same letter are not significantly different according to Fisher's LSD test ($p=0.05$).

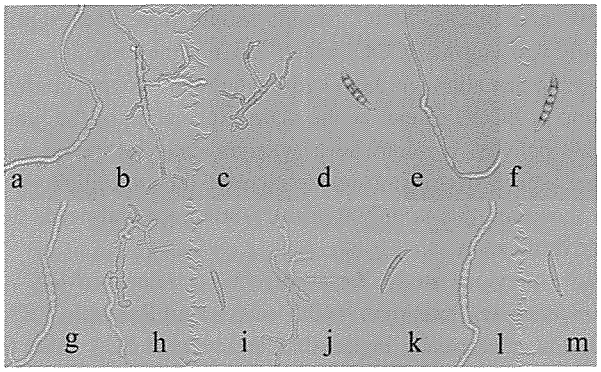

Fig. 3. Photomicrographs of conidia and germlings of *F. graminearum* exposured to MBC, BCOC, and BHC on agar for 8 hr at 25°C. Photographs marked with a–f refer to strain JT04 (MBC^{HR}) whereas g–m refer to strain 2021 (MBC^{S}). (a) Control of JT04, without fungicides; (b) JT04 and (treated with) 100 μg/mL MBC; (c) JT04 and 1.5 μg/mL BCOC; (d) JT04 and 5 μg/mL BCOC; (e) JT04 and 5 μg/mL BHC; (f) JT04 and 10 μg/mL BHC; (g) control of 2021, without fungicides; (h) 2021 and 1.0 μg/mL MBC; (i) 2021 and 5 μg/mL MBC; (j) 2021 and 1.5 μg/mL BCOC; (k) 2021 and 5 μg/mL BCOC; (l) 2021 and 5 μg/mL BHC; (m) 2021 and 10 μg/mL BHC.

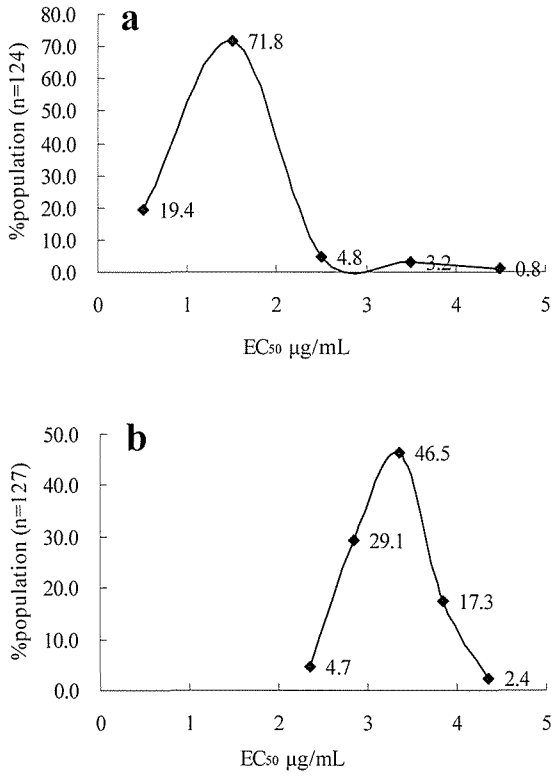

Fig. 4. Frequency distribution of sensitivity of 251 *F. graminearum* isolates to BCOC on PDA. (a) Field strains (124) with unknown sensitivity to MBC collected from Jiangsu Province, China in May 2010; (b) Field strains (127) collected from different provinces of China between 2000 and 2010 and determined to be resistant to MBC (MIC> 2 μg/mL).

conidia at their lethal concentrations (Fig. 3d, f, i, k, m).

3. *Baseline sensitivity to BCOC*

The EC_{50} values of strains with unknown sensitivity to MBC and collected from Jiangsu Province in May 2010 ranged from 0.92 to 4.26 μg/mL and averaged 1.29±0.61 μg/mL, representing a sensitivity range of 4.63 times, and were divided into 5 groups with the same range: 0–1, 1–2, 2–3, 3–4, 4–5 μg/mL (Fig. 4a). The EC_{50} values of MBC^{R} strains collected from different provinces in China between 2000 and 2010 ranged from 2.21 to 4.50 μg/mL and averaged 3.23±0.41 μg/mL, representing a sensitivity range of 2.04 times, and were also divided into 5 groups with the same range: 2.1–2.6, 2.6–3.1, 3.1–3.6, 3.6–4.1, 4.1–4.6 μg/mL (Fig. 4b). The frequency distribution of BCOC EC_{50} values was unimodal for both sets of *F. graminearum* populations, indicating that the data in Fig. 4a could be used as a baseline for monitoring the shift of sensitivity in *F. graminearum* populations to BCOC (Fig. 4).

4. *Efficacy of BCOC* in vivo

All of the inoculated plants in control plots were infected with disease intensity of 58.0%. Overall, control of FHB *in vivo* was better with BCOC than with MBC or BHC (Table 3). Although protective control of the susceptible strain was a little higher with BCOC than with MBC, there was no significant difference between them as expected. Protective control of the

Table 3. Control of FHB by BCOC (50% WP), MBC (50% WP), and BHC (33.5% SC) *in vivo* [a]

Fungicide	Dosage (μg/mL)	Protective disease control (%)[b]		Curative disease control (%)	
		2021[c]	JT04	2021	JT04
BCOC	800	87.5 a	72.4 a	73.5 a	71.8 a
BCOC	400	82.8 a	71.8 a	72.4 a	65.5 b
MBC	800	84.3 a	51.7 cd	62.1 b	49.8 d
MBC	400	81.4 a	48.3 d	58.6 b	46.7 d
BHC	800	55.2 b	58.6 b	48.3 c	58.6 c
BHC	400	46.7 b	55.7 bc	44.8 c	55.2 c

[a] On day 1, individual wheat plants were treated with fungicides or water to measure the protective control. Half of the plants were artificially inoculated on day 2 with a conidial suspension of either an MBC^{S} wild-type strain (2021) or an MBC^{R} wild-type strain (JT04) of *F. graminearum*. On day 3, the other half of the plants were treated with the same fungicides or water to measure curative control. [b] Percentage disease control is relative to inoculated plants that were treated with water rather than with fungicide. [c] Within a column, means followed by the same letter are not significantly different according to Fisher's LSD test (p=0.05).

resistant strain was significantly greater with BCOC than with MBC and curative control was always greater with BCOC than with the other two fungicides (Table 3). In addition, control of the sensitive strain was better than the resistant strain with BCOC and MBC. Although BHC is known as a preventive fungicide, it provided some curative activity here. This might mainly because the time between inoculation and treatment was too limited for the strains to infect.

Discussion

Fungicides with a similar structure tend to develop cross-resistance, as has been documented for the benzimidazole fungicides carbendazim, thiabendazole, and benomyl.[16,17] BCOC, MBC, and BHC also have structural similarities, and in this research we determined the efficacy of BCOC, MBC, and BHC against *F. graminearum in vitro* and in the field. Even though BCOC shares structural elements with MBC, inhibition of MBC^R strains was much greater with BCOC than with MBC. The efficacy of BCOC against MBC^S strains was almost equal to that of MBC and was much better than that of BHC, which also has some structural similarity to BCOC. However, the inhibition of mycelial growth of MBC^R strains was coincidentally a little worse than MBC^S strains for BCOC. These phenomena suggested an extremely low level of cross-resistance, indicating that the benzimidazole-2-carbamate component played a role in BCOC. Furthermore, if 8-oxyquinoline-copper was the main component responsible for inhibition by BCOC, inhibition by BHC should have been greater because BHC contains one more 8-oxyquinoline component than BCOC. We therefore hypothesize that the component in BCOC that was mainly responsible for its fungicidal activity was benzimidazole-2-carbamate. This inference was also supported by the appearance of germinated conidia, which was similar after treatment with BCOC and MBC. In other words, the activity of BCOC cannot be explained as resulting from the simple combination of MBC and BHC. Rather, the mechanism of BCOC seems to be similar to but also distinct from that of MBC.

In another of our studies, the results were similar with *Botrytis cinerea* and *Sclerotinia sclerotiorum* in that BCOC showed much better inhibition against MBC^R strain than MBC, and slightly lower efficacy against MBC^S strain than MBC; BHC acted less well than BCOC or MBC (unpublished data). Although the data were different, the tendency was the same for each pathogen. These results indicated that BCOC do have a somewhat similar mechanism to MBC and provided further proof of our hypothesis.

Previous studies demonstrated that mutations at codon 167 or 200 of the β_2-tubulin gene lead to MBC^{MR}, and a mutation at codon 198 alone or accompanied by a mutation at codon 73 leads to MBC^{HR}.[7,18,19] We assume that mutations at these codons alter the structure of β_2-tubulin protein and thereby reduce the interaction between the protein and the fungicide molecule. If the functional configuration had been similar for MBC and BCOC, MBC^R strains should have been significantly resistant to BCOC, which was not the case. It is also possible that although the 8-hydroxyquinilato-copper configuration in BCOC cannot be the main explanation of the fungicide's activity, it might have synergistically interacted with other aspects of the molecule to increase the molecule's activity; however, this is only a primary inference and needs further verification.

In a previous study, the average EC_{50} value of MBC^R *F. graminearum* strains for MBC was 9.2 ± 1.9 μg/mL.[5] That value is substantially higher than the average EC_{50} value for MBC^R *F. graminearum* strains for BCOC in the current study, which was 3.2 ± 0.4 μg/mL. The average EC_{50} value of MBC^S strains for MBC, however, was 0.6 ± 0.01 μg/mL in the previous study, [5] which was substantially lower than that for BCOC in the current study. Nevertheless, control of MBC^S and MBC^R strains by BCOC was excellent *in vivo*. In particular, the curative effect against inoculated FHB was much greater with BCOC than with MBC. A larger scale field experiment will be needed in further research.

Previous studies with BHC demonstrated that its fungicidal activity was not affected by organic materials, metals, chelating molecules, or pH fluctuation and that long-term exposure to BHC did not elicit resistance.[20] Determing whether BCOC has some of these same desirable characteristics will require additional research. Additional research is also needed to determine whether BCOC is safe for applicators and the environment and whether modification of radicals on its benzene ring will increase its fungicidal efficacy or otherwise alter its activity. The results reported here indicate that BCOC could be valuable for the synthesis of new fungicides for disease control in general and for management of fungicide resistance in particular.

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References

1) T. Ban and K. Suenaga: *Euphytica* **113**, 87–99 (2000).
2) X. L. Wang, Y. F. Cui, F. Y. Fan, Y. L. Song, J. P. Ren, Q. L. Meng and W. J. Xu: *J. Phytopathol.* **158**, 576–578 (2010).
3) C. J. Chen, J. X. Wang, Q. Q. Luo, S. K. Yuan and M. G. Zhou: *Pest Manag. Sci.* **63**, 1201–1207 (2007).
4) H. K. Li, Y. M. Diao, J. X. Wang, C. J. Chen, J. P. Ni and M. G. Zhou: *Crop Prot.* **27**, 90–95 (2008).
5) M. G. Zhou and J. X. Wang: *Acta Phytopathol. Sin.* **31**, 365–370 (2001).
6) Y. J. Zhang, J. J. Yu, Y. N. Zhang, X. Zhang, C. J. Chen, J. X. Wang, D. W. Hollomom, P. S. Fan and M. G. Zhou: *Mol. Plant-Microbe Interact.* **22**, 1143–1150 (2009).
7) C. J. Chen, J. J. Yu, C. W. Bi, Y. N. Zhang, J. Q. Xu, J. X. Wang and M. G. Zhou: *Phytopathoogy* **99**, 1403–1411 (2009).
8) N. Lisker: *Crop Prot.* **9**, 439–445 (1990).

9) N. Lisker and A. Meiri: *Crop Prot.* **11**, 155–159 (1992).
10) C. Revellin, P. Leterme and G. Catroux: *Biol. Fertil. Soils* **16**, 211–214 (1993).
11) G. Nicoletti, E. Domalewska and R. Borland: *Mycol. Res.* **103**, 1073–1084 (1999).
12) Y. Chen, H. K. Li, C. J. Chen and M. G. Zhou: *Phytoparasitica* **36**, 326–337 (2008).
13) S. M. Liu, Y. Chen, J. J. Yu, C. J. Chen, J. X. Wang and M. G. Zhou: *Pest Manag. Sci.* **66**, 482–489 (2009).
14) J. X. Wang, H. X. Ma, Y. Chen, X. F. Zhu, W. Y. Yu, Z. H. Tang, C. J. Chen and M. G. Zhou: *Crop Prot.* **28**, 882–886 (2009).
15) L. M. Reid, R. W. Nicol, T. Ouellet, M. Savard, J. D. Miller, J. C. Young, D. W. Stewart and A. W. Schaafsma: *Phytopathology* **89**, 1028–1037 (1999).
16) W. H. Chung, W. C. Chung, P. F. Ting, C. C. Ru, H. C. Huang and J. W. Huang: *J. Phytopathol.* **157**, 742–747 (2009).
17) P. Leroux, F. Chapeland, D. Desbrosses and M. Gredt: *Crop Prot.* **18**, 687–697 (1999).
18) J. A. Butters and D. W. Hollomon: *Pestic. Sci.* **55**, 501–503 (1999).
19) H. X. Li, Y. J. Lu, J. X. Wang and M. G. Zhou: *J. Nanjing Agric. Univ.* **25**, 41–44 (2002) (in Chinese, with English abstract).
20) G. Nicoletti, E. Domalewska and R. Borland: *Mycol. Res.* **103**, 1085–1097 (1999).

Fusarium graminearum に対する新規キレート殺菌剤カルベンダジム－8-オキシキノリン－銅の効果

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カルベンダジム（MBC）とオキシン-銅（BHC）のキレート化合物であるカルベンダジム－8-オキシキノリン－銅（BCOC）の効果を，MBC および BHC と比較したところ，インビトロでの *Fusarium graminearum* の MBC 耐性株に対する抑制効果では，MBC や BHC，またそれらの混合物より強く，MBC 高度耐性株に対する BCOC の最小抑制濃度は，MBC の 10 倍も低い値であった．発芽管のあらわれ方は，BCOC 処理と MBC 処理で類似していた．BCOC の EC_{50} は，2010 年に採集された 124 野生株に対しては 1.29±0.61 μg/mL であり，2000～2010 年に採集された 127 耐性株に対しては，3.23±0.41 μg/mL であった．圃場において，感受性株や抵抗性株を人工的に接種させた場合，BCOC はムギ赤かび病に対し優れた予防および治療効果を示した．BCOC の高い効果は，8-オキシキノリン－銅が分子構造を変化させ，それによって BCOC 中のカルベンダジムのラジカルと，*F. graminearum* 中の β_2-チューブリンタンパク質との間の相互作用を促進するためと思われる．