Dimethachlon Resistance in *Sclerotinia sclerotiorum* in China

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Abstract

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The dicarboximide fungicide dimethachlon has been widely used for controlling *Sclerotinia sclerotiorum* in China for more than a decade. To assess the current status of dimethachlon resistance in *S. sclerotiorum* in China, 2,424 isolates were collected from disease-infected oilseed rape and soybean plants in five provinces of China in 2011 and 2012, and dimethachlon resistance was monitored by mycelial growth inhibition method on potato dextrose agar (PDA) media. Dimethachlon at 5 µg/ml was used as a discriminatory dose to detect resistance in all isolates, and 50% effective concentration values were determined for all dimethachlon-resistant isolates and some sensitive isolates. No dimethachlon resistance was detected in isolates from Anhui province (eastern China), Gansu province (northwestern China), and Qinghai province (western China). In Hunan province (central China), 3 of 268 (1.12%) isolates collected from oilseed rape plants in 2012 were re-

sistant to dimethachlon, and the resistance ratios for the three resistant isolates were 4.56, 32.70, and 105.53, respectively. In Heilongjiang province (northeastern China), 8 of 243 (3.29%) isolates collected from soybean plants in 2011 were resistant to dimethachlon, with resistance ratios of 5.57 to 94.80; 11 of 409 (2.69%) isolates collected in 2012 were resistant to dimethachlon, with resistance ratios of 3.21 to 9.69. Cross-resistance studies showed that there was positive cross-resistance between dimethachlon and iprodione, procymidone, and the N-phenyl carbamate fungicide diethofencarb. No cross-resistance was found between dimethachlon and carbendazim, tebuconazole, kresoxim-methyl, thiram, and boscalid. Compared with the sensitive isolates of *S. sclerotiorum*, the field-dimethachlon-resistant isolates were more sensitive to osmotic pressure, grew more slowly on PDA media, and were less pathogenic on leaves of oilseed rape.

Sclerotinia sclerotiorum (Lib.) de Bary is one of the most devastating and economically important necrotrophic fungal phytopathogens. It is capable of infecting more than 400 species of plants worldwide (3), including numerous weeds and important crops such as soybean, sunflower, canola, and oilseed rape (28). Infections of oilseed rape plants by germinating ascospores of *S. sclerotiorum* occur initially on flowers or petals in spring. When the infected petals fall, many will adhere to leaves, stems, and early pods (1,7,33). *Sclerotinia* stem rot caused by *S. sclerotiorum* is severe in many countries, including Canada (2), the United States (4), Australia (19), and China (22,30,38). In China alone, the annual oilseed rape acreage is more than 7 million ha (34). Annual oilseed rape yield losses caused by *S. sclerotiorum* range from 5 to 30% and yield losses of severely infected fields are as high as 80% (12). Because breeding programs for disease-resistant oilseed rape have been hampered by limited genetic resources (21), *S. sclerotiorum* continues to impose serious limitations on oilseed rape production worldwide (35).

Application of fungicides is an important tool for controlling *S. sclerotiorum* (22). The benzimidazole fungicide carbendazim has been widely and extensively used against *S. sclerotiorum* for several decades in China (30,39). High levels of carbendazim resistance and reduced field efficacy have been reported (17,27,29,36,37). Widespread control failures due to carbendazim resistance resulted in a suspension of carbendazim application for control of *S. sclerotiorum* in the 2000s in eastern China, especially in Jiangsu province (27,30). Dimethachlon, a dicarboximide fungicide, has been widely used to control *Sclerotinia* stem rot in China for many years. In Jiangsu province, dimethachlon has been recommended to supersede carbendazim for the management of *Sclerotinia* stem rot since 2002 (17). Wide use of dimethachlon and other dicarboximide fungicides has raised concerns about re-

sistance issues in *S. sclerotiorum*. Furthermore, dimethachlon-insensitive isolates of *S. sclerotiorum* have been detected in oilseed rape fields in Jiangsu province (22). A previous study demonstrated that a high level of dimethachlon resistance in *S. sclerotiorum* could be induced by consecutive selection on dimethachlon-amended potato dextrose agar (PDA) media in a laboratory setting (22). Although a high level of resistance to dicarboximide fungicides has not yet been reported in field isolates of *S. sclerotiorum*, resistance to the dicarboximide fungicide iprodione has been detected in closely related plant pathogens such as *S. homoeocarpa* (15), *S. minor* (14), *Botrytis cinerea* (24), and *Monilinia fructicola* (9). Therefore, it is necessary to monitor dimethachlon resistance and to study the biological characteristics of field-resistant isolates of *S. sclerotiorum* once resistance has emerged. The objectives of this study were to (i) monitor dimethachlon resistance in *S. sclerotiorum* isolates collected from different provinces of China; (ii) explore cross-resistance patterns between dimethachlon and other widely used fungicides, including iprodione, procymidone, fludioxonil, carbendazim, diethofencarb, tebuconazole, kresoxim-methyl, thiram, and boscalid; and (iii) compare biological characteristics such as mycelial growth rate, sclerotial production on PDA, pathogenicity on host plants, and osmotic sensitivity between the dimethachlon-resistant isolates and the sensitive isolates.

Materials and Methods

Isolates of *S. sclerotiorum*. Isolates of *S. sclerotiorum* were collected from oilseed rape plants with symptoms of *Sclerotinia* stem rot in central China (Hunan province), eastern China (Anhui province), western China (Qinghai province), and northwestern China (Gansu province) and from diseased soybean plants in northeastern China (Heilongjiang province) in 2011 and 2012 (Fig. 1). In Anhui, Hunan, Heilongjiang, and Qinghai provinces, isolates were collected from more than 20 fields per province. In Gansu province, isolates were collected from 15 fields. The fields were separated from each other by more than 20 km. In each field, sclerotia were randomly collected from crop plants with symptoms of *Sclerotinia* stem rot. Sclerotia collected from the same diseased plant were regarded as one isolate. One dimethachlon-sensitive isolate (SXMG) and one dimethachlon-resistant isolate (SX2) col-

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lected from Shaanxi province were also used in this study for comparison.

Fungicides. All fungicides used in this study were technical-grade products. Dimethachlon (96.2%) was provided by Wenzhou Pesticide Co. Ltd. and dissolved in methanol to produce a 1,000- μ g/ml stock solution. Carbendazim (98.1%) was provided by Haili Guixi Chemical Co. Ltd. and dissolved in hydrochloric acid (HCl) at 0.1 mol/liter to produce a 1,000- μ g/ml stock solution. Iprodione (95.3%) and procymidone (98.0%) were provided by Heyi Agrochemical Co. Ltd. Tebuconazole (96.2%) was provided by Sheyang Huanghai Pesticide Chemical Co. Ltd. Diethofencarb (95.4%) was provided by Lanfeng Chemical Company. Kresoxim-methyl (96.6%) was provided by Gengyun Chemical Co. Ltd. Thiram (96.0%) was provided by Guanlong Agrochemical Co. Ltd. Boscalid (97.0%) was provided by Kangbaotai Fine-Chemical Co. Ltd. All fungicides, except for dimethachlon and carbendazim, were dissolved in acetone to produce 1,000- μ g/ml stock solutions.

Resistance determination. Resistance determination was performed according to Ma et al. (22), with a few modifications. Sclerotia of *S. sclerotiorum* were bisected, sterilized in 75% alcohol for 3 min, transferred into 1% sodium hypochlorite solution, sterilized for 3 min, rinsed in sterile distilled water for 30 s, and dried on sterilized filter paper. Halves of sclerotia were placed on PDA (200 g of potato, 20 g of agar, and 20 g of dextrose per liter of distilled water) plates. Plates were incubated for 2 days at 26°C in a growth chamber with a 12-h photoperiod. Dimethachlon at 5 μ g/ml was used as the discriminatory dose. Mycelial plugs (6 mm in diameter) cut from the fresh margins of 48-h-old colonies were transferred upside down onto PDA amended with dimethachlon at 5 μ g/ml, using fungicide-free PDA as the control. After 48 h of incubation at 26°C in a growth chamber, mycelial growth was measured in two perpendicular directions. Isolates that showed growth on dimethachlon at 5 μ g/ml were tentatively considered resistant, whereas the completely inhibited isolates were considered sensitive to dimethachlon (22). The percentage of resistant isolates was calculated as follows: percentage of resistant isolates (%) = total number of resistant isolates/total number of isolates detected $\times$ 100.

For all of the tentatively identified resistant isolates and some arbitrarily selected sensitive isolates, half maximal (50%) effective concentrations (EC_{50}) were determined as follows. PDA media amended with dimethachlon at 0, 1, 2, 4, 8, 16, 32, 64, and 128 μ g/ml were used for all resistant isolates. PDA media amended with dimethachlon at 0, 0.125, 0.25, 0.5, 1.0, and 2.0 μ g/ml were used for sensitive isolates. For cross-resistance determination, at least five concentrations of each fungicide were used for EC_{50}

assays. There were three replicates for each concentration and all tests were performed twice. Resistance ratio for the resistant isolate was calculated as follows: resistance ratio = EC_{50} value of resistant isolate/average EC_{50} value of sensitive isolates.

The average EC_{50} value of sensitive isolates was determined from 214 isolates sampled from the five provinces.

Osmotic sensitivity determination. Three field-dimethachlon-sensitive isolates (SXMG, GUMG, and HLJMG) and three field-dimethachlon-resistant isolates (HN1, HLJ4, and SX2) were used to determine osmotic sensitivity. For each isolate, mycelial plugs (6 mm in diameter) were taken from the margins of 48-h-old colonies and transferred onto PDA media amended with NaCl (at 0, 1.25, 2.5, 5.0, 10, 20, 40, and 80 g/liter) or glucose (at 0, 10, 20, 40, 80, 100, 150, and 200 g/liter). PDA free of NaCl and glucose was used as the untreated control. Six plates (replicates) for each concentration of NaCl and glucose were incubated at 26°C for 48 h in a growth chamber with a 12-h photoperiod. Diameters of fungal colonies were measured perpendicularly in millimeters. Percentage of mycelial radial growth inhibition relative to the NaCl- and glucose-free control was calculated. The experiment was performed twice.

Characterization of sclerotial production and mycelial growth on PDA. The same three sensitive and three resistant isolates used in the osmotic sensitivity experiment were characterized for their sclerotial production and mycelial growth on PDA media. For each isolate, mycelial plugs (6 mm in diameter) were taken from the margins of 48-h-old colonies and transferred onto PDA plates. Six plates for each isolate were incubated at 26°C for 21 days in a growth chamber with a 12-h photoperiod. The plates were observed every day. After 48 h of incubation, the diameter of each fungal colony was measured perpendicularly in millimeters. The start time for sclerotia formation (the number of days from incubation to initial formation of sclerotia) and the end time for sclerotia growth (the number of days from incubation to the end of growth of sclerotia) on PDA plates were recorded. At the end of 21 days of incubation, the number and distribution of sclerotia on PDA plates were recorded. All sclerotia in each dish were weighed after drying at 50°C for 2 days.

Determination of pathogenicity on detached leaves of host plants. Three field-dimethachlon-resistant isolates and four sensitive isolates were used for pathogenicity tests on oilseed rape plants. Leaves of similar growth position and size were detached from oilseed rape plants, rinsed with sterile water, air dried in a transfer hood, transferred to 15-cm petri dishes lined with wet filter paper to maintain high humidity, and then inoculated on the adaxial surface with mycelial plugs (6 mm in diameter) cut from the margins of 48-h-old colonies of *S. sclerotiorum* isolates. A mycelial plug of a sensitive isolate and another plug of a resistant isolate were placed on opposite sides of the midrib. The inoculated leaves were incubated in a growth chamber (26°C, photoperiod of 16 h). Lesions were measured after 24, 48, and 72 h of incubation. The experiment was performed twice, with at least six replicates for each isolate.

Data analysis. EC_{50} values were calculated by the Bioassay Analysis for Quantity Data procedure in Data Processing System (ver. 7.05; Hangzhou RuiFeng Information Technology Co. Ltd.). The analysis of variance procedure in Statistical Product and Service Solutions (ver. 17.0; SPSS Inc.) and Fisher's least significant difference test ($P = 0.05$) were used to test for significant differences in biological characteristics such as mycelial growth rate, sclerotial production, pathogenicity, and osmotic sensitivity among different isolates. Graphs were created in Microsoft Excel (ver. 2010; Microsoft Corporation).

Results

Dimethachlon resistance in field isolates of *S. sclerotiorum* in China. Dimethachlon resistance was investigated in a total of 2,424 field isolates of *S. sclerotiorum* collected in 2011 and 2012. Only 22 isolates were resistant to dimethachlon, and the overall resistance frequency (percentage of resistant isolates) was 0.91%,

Fig. 1. Map of China. Letters A, B, C, and D represent Anhui, Hunan, Gansu, and Qinghai provinces, respectively, where isolates of *Sclerotinia sclerotiorum* were collected from oilseed rape plants with symptoms of Sclerotinia stem rot. Letter E represents Heilongjiang province, where isolates of *S. sclerotiorum* were collected from soybean plants.

indicating that the development of dimethachlon resistance in *S. sclerotiorum* was still at the initial stage (Table 1). Of the five provinces where field isolates were collected, dimethachlon-resistant isolates were detected only from Hunan province and Heilongjiang province. One isolate with a resistance ratio as high as 105.53 was collected from Hunan province.

Spectrum of cross-resistance in field-resistant isolates. There was positive cross-resistance among the dicarboximide fungicides dimethachlon, iprodione, and procymidone, which share the same

mode of action (Table 2). Positive cross-resistance was also found between the dicarboximide fungicides and the carbamate fungicide diethofencarb, whose mode of action is different from that of the dicarboximide fungicides. The target of diethofencarb is β -tubulin assembly in mitosis, whereas the target of the dicarboximide fungicides is mitogen-activated protein (MAP)/histidine kinase in osmotic signal transduction. No cross-resistance was found between the dicarboximide fungicides and other fungicides such as carbendazim, tebuconazole, kresoxim-methyl, boscalid, and thiram.

Table 1. Dimethachlon resistance in field isolates of *Sclerotinia sclerotiorum* in five provinces of China^v

Province	Host	Year	Monitored (<i>n</i>) ^w	Resistant (%) ^x	Median (minimum–maximum)	
					EC ₅₀ (μg/ml) ^y	Resistance ratio ^z
Hunan	Oilseed rape	2011	151	0	—	—
		2012	268	3 (1.12)	9.69 (1.35–31.27)	32.70 (4.56–105.53)
Anhui	Oilseed rape	2011	227	0	—	—
		2012	336	0	—	—
Qinghai	Oilseed rape	2011	314	0	—	—
		2012	—	—	—	—
Gansu	Oilseed rape	2011	55	0	—	—
		2012	421	0	—	—
Heilongjiang	Soybean	2011	243	8 (3.29)	9.96 (1.65–28.09)	33.61 (5.57–94.80)
		2012	409	11 (2.69)	1.69 (0.95–2.87)	5.70 (3.21–9.69)

^v Symbol: — indicates no data available or could not be calculated.

^w Number of isolates monitored.

^x Number and frequency (%) of resistant isolates. Resistance frequency (%) = number of resistant isolates/number of isolates monitored × 100.

^y Half maximal (50%) effective concentrations (EC₅₀) values of resistant isolates.

^z EC₅₀ value of resistant isolate/average EC₅₀ value of sensitive isolates. The average EC₅₀ value of sensitive isolates was 0.2963 μg/ml.

Table 2. Cross-resistance to different fungicides in field-resistant isolates of *Sclerotinia sclerotiorum*

Fungicides		Dimethachlon EC ₅₀ values (μg/ml) ^z					
		Dimethachlon-resistant isolates			Field-sensitive isolates		
Fungicide group	Common name	HN1	HLJ4	SX2	SXMG	HLJMG	GSMG
Dicarboximide	Dimethachlon	31.27	28.10	29.57	0.38	0.22	0.23
	Iprodione	> 40	> 40	26.28	0.15	0.26	0.13
	Procymidone	> 40	> 40	> 40	0.23	0.21	0.23
Phenylpyrrole	Fludioxonil	0.0012	1.08	0.0010	0.0012	0.0013	0.0013
Benzimidazole	Carbendazim	0.06	0.99	0.06	0.07	0.05	0.07
Carbamate	Diethofencarb	19.49	35.64	22.57	0.51	0.36	0.52
Triazole	Tebuconazole	0.50	0.91	0.51	0.22	0.17	0.05
Strobilurin	Kresoxim-methyl	0.90	0.92	0.76	0.29	0.07	0.15
Organo-sulfur	Thiram	0.66	1.65	0.34	1.2	0.92	0.89
Pyridine-carboxamide	Boscalid	0.11	0.09	0.22	0.06	0.02	0.06

^z EC₅₀ = half maximal (50%) effective concentrations of resistant isolates. Dimethachlon-resistant isolates HN1, HLJ4, and SX2 were collected from Hunan, Heilongjiang, and Shaanxi provinces, respectively. Field-sensitive isolates SXMG, HLJMG, and GSMG were collected from Shaanxi, Heilongjiang, and Gansu provinces, respectively.

A. Sensitivity to glucose

B. Sensitivity to NaCl

Fig. 2. Comparison of osmotic sensitivity between sensitive isolates (SXMG, GSMG, and HLJMG) and dimethachlon-resistant isolates (HN1, HLJ3, and HLJ4). Isolates SXMG, GSMG, and HN1 were collected from Shaanxi, Gansu, and Hunan provinces, respectively. Isolates HLJMG, HLJ3, and HLJ4 were collected from Heilongjiang province. Data represent the means of mycelial growth inhibition ± standard error (SE) of at least six replicates for each treatment. **A**, Sensitivity to glucose; **B**, sensitivity to NaCl.

Osmotic sensitivity. The field-dimethachlon-resistant isolates grew more slowly than field-sensitive isolates on PDA amended with NaCl (lower than 40 g/liter) or glucose (lower than 200 g/liter), indicating that the field-resistant isolates were more sensitive to osmotic pressure (Fig. 2).

Mycelial growth rate and sclerotial production on PDA. Compared with the field-sensitive isolates, there was significantly ($P < 0.001$) slower mycelial growth on PDA for the dimethachlon-resistant isolates (Table 3). No definite conclusion could be drawn about differences in the amount and dry weight of sclerotia between the dimethachlon-resistant isolates and the sensitive isolates. It was obvious that the start time and end time for sclerotial production in all three of the resistant isolates was delayed compared with the sensitive isolates.

Pathogenicity on detached leaves of host plants. Pathogenicity of the field-dimethachlon-resistant isolates was significantly lower ($P < 0.001$) than that of the sensitive isolates (Table 4). Resistant isolates SX2 and HLJ3 completely lost pathogenicity on detached leaves of oilseed rape. Lesion diameters and disease incidences of the resistant isolates were much lower than those of the sensitive isolates, especially at 72 h. Furthermore, the speed of mycelial invasion into plant tissue, as measured by lesion diameter at the first 24 h, decreased obviously for the resistant isolates. Actually, no lesion symptoms were observable with the naked eye after the first 24 h for the resistant isolates, whereas lesion symptoms were apparent for the sensitive isolates.

Discussion

Field isolates of *S. sclerotiorum* with reduced sensitivity to dimethachlon were detected in Jiangsu province in 2006 and 2007 (22). Medium to high levels of resistance to dimethachlon have been detected in our laboratory in samples from Shaanxi province (data not shown). In the adjacent Gansu province, no resistant

isolate was detected. The geographic distribution of resistant isolates could not be explained by the intensity of dimethachlon application. The benzimidazole fungicide carbendazim and the dicarboximide fungicides dimethachlon, procymidone, and iprodione; the triazole fungicide tebuconazole; and the imidazole fungicide prochloraz were used to control Sclerotinia rot in oilseed rape and soybean fields in China. Dimethachlon has been one of the major fungicides in controlling Sclerotinia stem rot in oilseed rape after carbendazim resistance became widespread in eastern China (22,30). However, no resistant isolate was found in Anhui province. In Hunan province and Heilongjiang province, where use of dimethachlon was not as extensive as in eastern China (20), dimethachlon resistance was detected and some isolates even evolved high resistance to dimethachlon. In general, dimethachlon resistance frequencies were still low in China and the resistance seemed to be still at the “emergence phase” stage of the development of fungicide resistance (5). High resistance levels with resistance ratios greater than 50 for some isolates from Hunan and Heilongjiang provinces demonstrated that, even at the initial stage of resistance development, high resistance could occur in field conditions. At this stage of resistance development, does dimethachlon resistance in *S. sclerotiorum* develop faster in regions where dimethachlon is extensively applied than in regions with lower levels of application? There are still debates about the effects of fungicide application rate on the development of resistance. Some scientists believe that, compared with high rates of fungicide application, low rates may hasten the evolution of resistance by increasing mutation frequencies (11). Our results indicated that resistance emergence could not be inferred from the intensity of fungicide application, at least during the initial stage of the development of dimethachlon resistance in *S. sclerotiorum*.

Positive cross-resistance was observed among the dicarboximide fungicides dimethachlon, iprodione, and procymidone. This result

Table 3. Comparison of mycelial growth and sclerotial production on potato dextrose agar (PDA) plates between field-resistant and sensitive isolates of *Sclerotinia sclerotiorum*^a

Isolates (EC ₅₀) ^x	Mycelial growth (cm) ± SE ^y	Sclerotia formation time (day) ^w		Number of sclerotia/dish	Sclerotia dry weight (g) ^z
		Start time	End time		
HLJMG (0.22)	3.60 ± 0.04 d	5 a	8 a	19.5 d	0.1144 c
GSMG (0.23)	2.80 ± 0.20 c	5 a	8 a	16.0 c	0.1228 c
SXMG (0.38)	3.30 ± 0.18 cd	5 a	8 a	12.8 b	0.0519 b
HLJ3 (25.32)	1.70 ± 0.05 a	11 c	15 c	10.3 a	0.0156 a
HLJ4 (28.10)	1.90 ± 0.09 ab	9 b	12 b	10.3 a	0.0839 b
HN1 (31.27)	2.10 ± 0.14 b	9 b	12 b	19.5 d	0.0722 b

^a A different letter in the same column indicates that the difference was significant according to Fisher's least significant difference test ($P = 0.05$) in SPSS.

^w Sclerotial production start time was the number of days from incubation to the initial formation of sclerotia. The end time was the number of days from incubation to the end of growth of sclerotia on a PDA plate.

^x Half maximal (50%) effective concentration (EC₅₀) values for dimethachlon. Isolates HLJMG, GSMG, and SXMG were dimethachlon-sensitive isolates collected from Heilongjiang, Gansu, and Shaanxi provinces, respectively. The dimethachlon-resistant isolates HLJ3 and HLJ4 were collected from Heilongjiang province and HN1 was collected from Hunan province.

^y SE = standard error.

^z Dry weight of sclerotia was the weight of all sclerotia in a PDA plate.

Table 4. Comparison of pathogenicity on detached leaves of oilseed rape plants between field-resistant and sensitive isolates of *Sclerotinia sclerotiorum*^a

Isolates (EC ₅₀) ^x	24 h		48 h		72 h	
	LD (cm) ± SE	DI (%)	LD (cm) ± SE	DI (%)	LD (cm) ± SE	DI (%)
GSMG (0.23)	0.85 ± 0.03 a	50.0	2.14 ± 0.09 c	62.5	3.01 ± 0.32 b	75.0
SXMG (0.38)	0.78 ± 0.05 a	37.5	2.06 ± 0.12 c	100.0	3.31 ± 0.12 b	100.0
HLJMG (0.22)	0.94 ± 0.06 a	87.5	2.34 ± 0.16 c	100.0	3.52 ± 0.17 b	100.0
HLJ3 (25.32)	—	—	—	—	—	—
HLJ4 (28.10)	—	—	1.65 ± 0.05 b	12.5	1.38 ± 0.40 a	37.5
HN1 (31.27)	—	—	0.85 ± 0.11 a	20.0	1.20 ± 0.02 a	20.0
SX2 (29.57)	—	—	—	—	—	—

^a LD = lesion diameter, SE = standard error, and DI = disease incidence (number of successful infections/number of total inoculations × 100); — indicates no infection and, therefore, no data available. The same letter in the same column indicates that the difference was not significant according to Fisher's least significant difference test ($P = 0.05$) in SPSS.

^x Half maximal (50%) effective concentration (EC₅₀) values. Isolates GSMG, SXMG, and HLJMG were dimethachlon-sensitive isolates collected from Gansu, Shaanxi, and Heilongjiang provinces, respectively. The dimethachlon-resistant isolates HLJ3 and HLJ4 were collected from Heilongjiang province, and HN1 and SX2 were collected from Hunan province and Shaanxi province, respectively.

was not surprising because these three fungicides have almost the same mode of action: acting on MAP/histidine kinase in osmotic signal transduction (18,24). However, positive cross-resistance between the dicarboximide fungicides and diethofencarb was also detected. The mode of action of diethofencarb is the inhibition of β -tubulin assembly during mitosis (25). Positive cross-resistance between the two groups of fungicides with totally different modes of action could have been caused by a multi-drug-resistance mechanism such as the ATP-binding cassette, as reported in *B. cinerea* (16). Field isolates of *B. cinerea* resistant to both procymidone and diethofencarb have been reported but not all of the procymidone-resistant isolates were also resistant to diethofencarb (31), suggesting that procymidone resistance and diethofencarb resistance evolved independently in *B. cinerea*.

Previous studies showed that laboratory-induced dicarboximide-resistant isolates of *B. cinerea* (6) and *Alternaria brassicicola* (13,23) exhibited greater osmotic sensitivity and decreased virulence on hosts, whereas field-resistant isolates did not show increased osmotic sensitivity and retained high virulence on plants. In contrast, our results showed that the field-dimethachlon-resistant isolates of *S. sclerotiorum* were more sensitive to osmotic pressure, less virulent on the host plants oilseed rape, grew more slowly on PDA, and showed delayed sclerotia formation compared with the sensitive isolates. Colony characteristics such as mycelial radial growth rates of *S. sclerotiorum* in vitro have been related to pathogenicity under controlled environmental conditions (8,40). However, studies on *S. sclerotiorum* isolates from Australia showed that there was no correlation between pigmentation or colony diameter on PDA with pathogenicity as measured by diameter of cotyledon lesions on the host *Brassica napus* (10). Genetic diversity studies on *S. sclerotiorum* demonstrated that genetic diversity was not related to the pathogenicity of the pathogen (26,32). The present results indicated that the field-dimethachlon-resistant isolates had lower fitness to compete with the field-sensitive isolates under natural field conditions. This may have been one of the reasons for the slow development of dimethachlon resistance under field conditions and the low frequency of resistant isolates after many years of dimethachlon application. Due to the present resistance status, dimethachlon resistance in field isolates of *S. sclerotiorum* should be closely monitored in the next several years, and integrated pest management strategies such as biological, bio-technical, and cultural methods should be combined with chemical methods for controlling this devastating phytopathogen.

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