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Baseline sensitivity and control efficacy of propiconazole against *Sclerotinia sclerotiorum*

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ABSTRACT

Sclerotinia sclerotiorum, the causal agent of Sclerotinia stem rot (SSR) of oilseed rape, is a broad-host-range pathogen. The demethylation inhibitor (DMI) fungicide propiconazole was officially approved in China in 2014 for control of this pathogen. In this study, baseline sensitivity of *S. sclerotiorum* to propiconazole was established. The distribution of the 50% effective concentration (EC_{50}) values was unimodal and fitted a normal distribution, but skewed to the right. The mean EC_{50} values for isolates collected in 2008 and 2014 were 0.338 ± 0.174 μg/mL (mean ± standard deviation, SD) and 0.315 ± 0.135 μg/mL (mean ± SD), respectively. There was no significant difference ($P = 0.375$) in EC_{50} values between 2008 and 2014. There was a significant correlation ($P < 0.001$, $r^2 = 0.507$) in sensitivity between propiconazole and tebuconazole, whereas no significant correlation ($P = 0.297$, $r^2 = 0.039$) could be detected between propiconazole and boscalid. Preventive efficacy of propiconazole for SSR was significantly ($P < 0.05$) higher than that of carbendazim. Propiconazole at 0.1 and 0.4 μg/mL significantly ($P < 0.001$) reduced the number but slightly increased the weight of sclerotia produced on potato dextrose agar (PDA) medium. Propiconazole significantly ($P < 0.05$) increased mycelial cell membrane permeability and mannan content of *S. sclerotiorum*. Light microscopic observations showed that propiconazole caused hyphal tips to branch more and hyphae thinner than the nontreated control.

1. Introduction

Sclerotinia sclerotiorum (Lib.) de Bary is a necrotrophic fungal pathogen capable of infecting more than 400 plant species worldwide, most of which are dicotyledons (Boland and Hall, 1994). Many agriculturally important crops, such as soybean, sunflower, peanut, and oilseed rape, can be infected by this pathogen during growing seasons, and the resulting diseases are widely known as Sclerotinia stem rot (SSR) or white mold (Bardin and Huang, 2001; Bolton et al., 2006). SSR may become a disastrous threat to rapeseed production, especially if the relative humidity is high during the flowering season. Rapeseed yield losses caused by SSR usually range from 10 to 20% in China and may be up to 80% for severe SSR outbreaks seasons (Li et al., 2006). The control of SSR has been relying heavily on fungicides because no highly resistant cultivars are available (Bolton et al., 2006; Wang et al., 2014).

In China, the earliest and most widely used fungicide for control of SSR is the methyl benzimidazole carbamate carbendazim. The mode of action (MoA) of carbendazim is to inhibit β-tubulin assembly in mitosis, and positive cross-resistance is common among members of the methyl benzimidazole carbamate class (FRAC, 2018). Fungicides with a single-site MoA are prone to resistance development, and resistance to carbendazim has been reported in numerous fungi such as S sclerotiorum (Wang et al., 2014; Zhu et al., 2016) and *Botrytis cinerea* (Saito et al., 2016; Konstantinou et al., 2015). Fungicide Resistance Action Committee (FRAC, 2018) categorizes carbendazim as at high risk for resistance development. Carbendazim resistance in *S. sclerotiorum* began to be reported in the late 1990s in eastern China (Pan, 1998; Shi et al., 2000). Recent studies demonstrate that resistance of *S. sclerotiorum* to carbendazim is still confined mainly to eastern China (Wang et al., 2014; Zhu et al., 2016). In 2003, the dicarboximide fungicide dimethachlone was registered in China to control SSR. Unfortunately, after only several years of applications, reduced sensitivity and low frequencies of resistance to dimethachlone occurred in the field and, for some isolates, resistance ratios have reached more than 100-fold (Ma et al., 2009; Zhou et al., 2014).

Fungicides with different MoAs are valuable tools for controls of plant pathogens and resistance management (Brent and Hollomon, 2007). As *S. sclerotiorum* has developed high levels of resistance to carbendazim and dimethachlone, it is important and imperative to ensure alternative fungicides with different MoAs than the methyl benzimidazole carbamate and dicarboximide fungicides available to farmers for control of SSR. Demethylation inhibitors (DMIs) comprise a

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large number of commercially very successful fungicides and are well known for their high effectiveness, broad-spectrum activity, and low risks for resistance development (Becher and Wirsel, 2012; Ziogas and Malandrakis, 2015). The DMI fungicide propiconazole was first registered to control SSR in 2014 in China (http://www.chinapesticide.org.cn/hysj/index.jhtml). Before a fungicide is registered or commercially available for control of a pathogen, baseline sensitivity of the target pathogen to the fungicide should be established as a reference point for future resistance monitoring programs (Russell, 2004). Therefore, baseline sensitivity of *S. sclerotiorum* to propiconazole should be constructed. Before the registration of propiconazole, three other DMI fungicides, i.e., triadimefon, tebuconazole, and prochloraz, had been registered in China for control of SSR in 2003, 2006, and 2007, respectively (http://www.chinapesticide.org.cn/hysj/index.jhtml). To evaluate the potential influence of possible exposure to triadimefon, prochloraz, and tebuconazole on the sensitivity of *S. sclerotiorum* to propiconazole, 75 isolates collected in 2008, the earliest isolates we have, as well as another set of 75 isolates sampled in 2014, were used to establish the baseline sensitivity to propiconazole. The objectives of the present study were to (i) construct baseline sensitivity of *S. sclerotiorum* to propiconazole, (ii) assay control effectiveness of propiconazole against SSR, and (iii) determine effects of propiconazole on hyphal morphology and cell membrane permeability of *S. sclerotiorum*.

2. Materials and methods

2.1. Sample collection and cultivation of S. sclerotiorum

Seventy-five isolates of *S. sclerotiorum* were collected in 2008 and another set of 75 isolates were sampled in 2014, from oilseed rape fields of 9 counties in Anhui Province of eastern China (Fig. 1). The oilseed rape fields were at least 20 km away from each other. Oilseed rape plants with typical symptoms of SSR were arbitrarily selected in the field, and the mature sclerotia collected from the stem of one plant were considered as one isolate. After being air-dried, sclerotia of each isolate were put into 0.2-mL centrifuge tubes and stored at 4 °C until needed for subsequent experiments. To cultivate the isolates, sclerotia were bisected, sterilized in 75% alcohol for 3 min, in 1% sodium hypochlorite solution for another 3 min, rinsed in sterile distilled water for 30 s, and air-dried on sterilized filter paper for about 30 min. Halves of the sclerotia were placed on potato dextrose agar (PDA) plates. Plates were incubated in the dark at 23 °C for 2 d in a growth chamber. PDA plugs (5 mm in diameter) were cut from the fresh margin of colonies and used as the inocula for next experiments.

2.2. Fungicides

Technical grade propiconazole (97.0% active ingredient [a.i.]) and boscalid (97.0% a. i.) were bought from Hubei Kang Bao Tai Fine-chemical Co. Ltd (Hubei Province, China). Technical grade tebuconazole (96.0% a. i.) was provided by Jiangsu Huanghai Pesticide Co. Ltd (Jiangsu Province, China). Stock solutions of these fungicides at 2000 μg/mL were prepared in acetone. Technical grade carbendazim (98.1% a. i., Tian Jin Jin Bei Chemical Co. Ltd) was dissolved in hydrochloric acid (HCl, 0.1 mol/L) to produce a stock solution at 2000 μg/mL. These stock solutions were stored at 4 °C for no longer than two weeks before being serially diluted for subsequent experiments.

2.3. Construction of baseline sensitivity of S. sclerotiorum to propiconazole

Baseline sensitivity of *S. sclerotiorum* to propiconazole was constructed based on the distribution of the 50% effective concentration (EC_{50}) values, as described by Liang et al. (2015a,b). EC_{50} values were determined based on mycelial growth inhibitions on PDA amended with propiconazole. A stock solution of propiconazole was serially diluted in acetone to a series of concentrations of 400, 200, 100, 50, and 25 μg/mL. Propiconazole dilutions were added to autoclaved PDA medium after the medium cooled down to about 50 °C, at a volume ratio of 1:1000. PDA plates amended with acetone at 0.1% by volume were used as the nontreated control. Mycelial plugs (5 mm in diameter) were cut from actively growing margins of 2-day-old colonies of each isolate and placed onto the center of PDA plates amended with fungicide or acetone. PDA plates were incubated at 23 °C in the dark for 48 h, and the diameter of each colony was measured twice in two perpendicular directions. EC_{50} values were calculated by the Probit procedure in the software Statistical Product and Service Solutions (SPSS, ver. 20, IBM Corporation, Chicago, IL, USA) according to Li et al. (2015). The profile of frequency distribution of EC_{50} values was used as the sensitivity baseline of *S. sclerotiorum* to propiconazole.

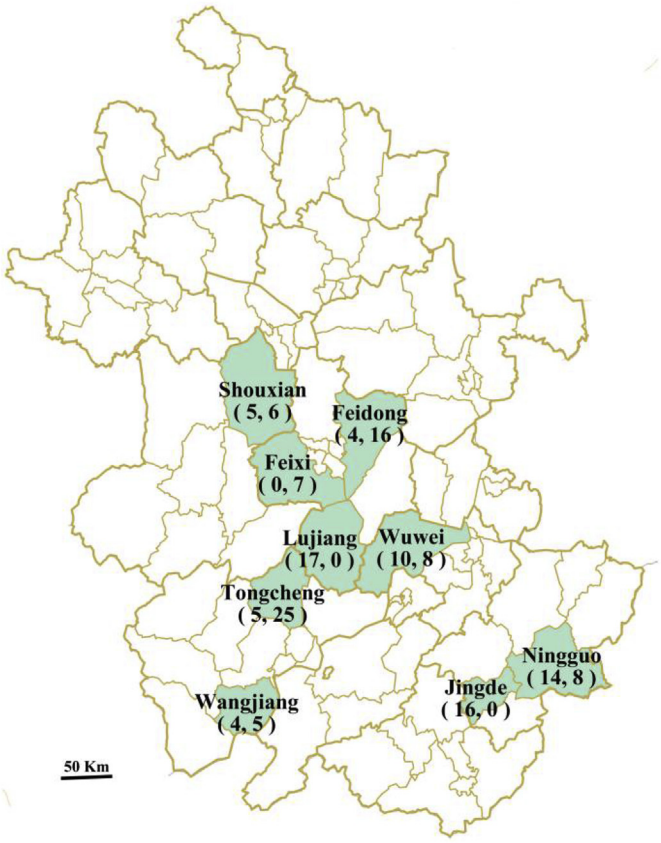

Fig. 1. Map of Anhui Province and geographic origins of *Sclerotinia sclerotiorum* isolates collected for the construction of baseline sensitivity. The two Arabic numerals separated by a comma in each parenthesis represent the number of isolates collected in 2008 and 2014, respectively.

2.4. Preventive and curative efficacy of propiconazole against S. sclerotiorum on potted oilseed rape plants

The method for determining the preventive and curative efficacy was according to Lu et al. (2015). Oilseed rape plants (cultivar Zhongshuang-10) were grown in plastic pots at 25 °C in growth chambers for 30 days. Stock solutions of propiconazole and the reference fungicide carbendazim were diluted with 0.1% Triton X-100 in water to the final concentrations of 80, 40, 20, and 10 μg/mL. Dilutions of propiconazole and carbendazim were sprayed on oilseed rape plants until run-off with a handheld sprayer (Xinmeir Co. Ltd., Zhejiang, China). Plants sprayed with 0.1% Triton X-100 in water were used as the nontreated control. For determination of preventive efficacy, the spayed oilseed rape plants were air-dried for 1 h and then inoculated on the adaxial surface with inverted mycelial plugs of the arbitrarily selected isolates ZFX3 and WW34. The inoculated plants were incubated