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Activity of the dinitroaniline fungicide fluazinam against *Bipolaris maydis*

Ya-Li Chen, Xue-Wei Mao, Jian-Xin Wang, Luo-Yu Wu, Ming-Guo Zhou, Yi-Ping Hou*

College of Plant Protection, Nanjing Agricultural University, Nanjing, Jiangsu 210095, China

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ABSTRACT

Fluazinam is a dinitroaniline fungicide with broad-spectrum activities. However, the activity of fluazinam against *Bipolaris maydis* which is the causal agent of southern corn leaf blight is unknown yet. In this study, baseline sensitivity of *B. maydis* to fluazinam was determined using 92 isolates collected during 2015 and 2016 from different geographical regions in Jiangsu Province of China, and the EC_{50} values ranged from 0.0396 to 0.9808 μg/ml with average value of 0.3853 ± 0.2297 μg/ml, and 0.079 to 0.7832 μg/ml with average value of 0.3065 ± 0.1384 μg/ml for mycelial growth and conidium germination respectively. Fluazinam did not affect the distribution of cell nucleus and the formation of septum of *B. maydis*. However, fluazinam could make mycelium of *B. maydis* contorted and the mycelial branches increased and inhibit the development of conidia. The result of transmission electron microscope showed that fluazinam damaged cell wall and cell membrane of mycelium, and make organelles in mycelial cell dissolved and vacuolated, and the cell almost broke up, which caused the intracellular plasma leakage increase. The protective activity test of fluazinam suggested that fluazinam had great control efficiency against *B. maydis* on detached corn leaves. Application of fluazinam at 10 μg/ml and 20 μg/ml, the control efficacy reached to 87.70% and 98.25% respectively. However, fluazinam had no curative activity against *B. maydis* on detached corn leaves. These results will contribute to us on evaluating the potential of the dinitroaniline fungicide fluazinam for management of diseases caused by *B. maydis* and understanding the mode of action of fluazinam against *B. maydis*.

1. Introduction

Maize (*Zea mays* L.) is not only one of the main grain crops but also an important feed crop and industrial raw material [1–4]. Many factors can reduce its production, such as diseases, pests, weeds, *etc*. Several maize diseases have been reported in various geographies [5–7]. Southern corn leaf blight (SCLB) caused by the filamentous fungus *Bipolaris maydis* (=*Cochliobolus heterostrophus*) is an important foliar disease of maize crop and often occurs in warm and humid areas [8,9]. The disease commonly came up in all countries around the world. As early as 1925, there had been varying degrees of occurrence [7,10]. This disease prevailed severely in the United States Corn Belt in 1970, lossing about 1 billion dollars [11,12]. In addition, all place have occurred in China, causing millions of losses [13]. With the development of resistant cultivars, the occurrence and harm of SCLB is basically controlled [14–16]. But due to the large area of single planting resistant varieties and the global climate warming, SCLB is still serious occurrence [13].

Traditionally, broad-spectrum fungicides were widely used to control SCLB, such as propiconazole, chlorothalonil and mancozeb [17,18]. However, the sensitivity of *B. maydis* to chlorothalonil and mancozeb decreased due to long-term large-scale use of a single fungicide [18]. In order to control SCLB preferably, it is necessary to find alternative fungicides to reduce the risk of fungicide resistance development.

The dinitroaniline fungicide fluazinam ([3-chloro-*N*-(3-chloro-5-trifluoromethyl-2-pyridyl)-α, α, α-trifluoro-2, 6-dinitro-p-toluidine]) is a highly effective fungicide with broad-spectrum activities [19]. The chemical structure of fluazinam is shown in Fig. 1. Although the mode of action is not fully understood, fluazinam is believed to be interrupting the fungal cell's energy production by uncoupling mitochondrial oxidative phosphorylation [20–22]. Fluazinam was firstly registered by Environmental Protection Agency (EPA) for agricultural uses. Previous studies showed that fluazinam could effectively control plant diseases caused by *Botrytis*, *Alternaria*, *Sclerotinia*, *Phytophthora* and *Colletotrichum* [23–26]. In China, the fungicide fluazinam was registered for controlling the plant diseases of potato late/early blight, tomato late blight, pepper blight, pepper anthracnose, root swelling of Chinese cabbage, gray mold of tomato, and brown patch of apple with different formulations of suspension concentrate, wettable powder and water dispersible granule (http://www.icama.org.cn/hysj/index.jhtml).

In recent study, we found that fluazinam had strong effect on

* Corresponding author.
E-mail address: houyiping@njau.edu.cn (Y.-P. Hou).

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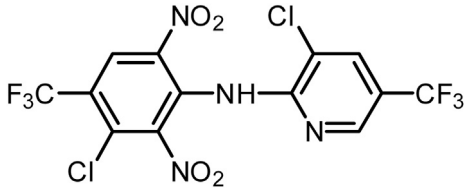

Fig. 1. The chemical structure of fluazinam.

mycelial growth of *B. maydis in vitro*, which indicated that fluazinam may be an potential fungicide for controlling SCLB. The effects of fluazinam on *B. maydis in vitro* have not been reported. Fluazinam has not been registered to control SCLB in China. In this study, the activity of fluazinam against *B. maydis* was evaluated, which can provide a scientific basis for effective control of SCLB. Therefore, the objectives of this study were to (i) establish the baseline sensitivity (sensitivity before exposure to the fungicide) to fluazinam in *B. maydis* populations from different maize fields in Jiangsu Province of China; (ii) investigate the effect of fluazinam on physiological characteristics of *B. maydis*; (iii) test the protective and curative activity of fluazinam against *B. maydis* on leaves of corn. This could provide new reference data for the management of SCLB and will increase our understanding the mode of action of fluazinam against *B. maydis* and other phytopathogens.

2. Materials and methods

2.1. Collection of B. maydis isolates

Maize plants with typical symptoms of *B. maydis* infection were collected from Jiangsu Province of China between 2015 and 2016. Maize leaf lesions were cut into pieces (5 × 5 mm), disinfected in 1% NaClO for 3 min, rinsed three times with sterile water, and placed on potato dextrose agar (PDA) medium plates amended with 100 μg/ml streptomycin sulfate. After 4 days of incubation at 25 °C, *B. maydis* was isolated from the edge of the colony and then transferred to a fresh PDA plate. All of the isolates were maintained on PDA slants and stored at 4 °C.

2.2. Fungicide and culture media

Technical grade fluazinam (active ingredient 97%; Jiangsu Lanfeng Bio Chemical Limited Co. Ltd) was dissolved in methanol at 1×10^4 μg/ml and kept as a stock solution. PDA medium was made from 200 g potato, 20 g dextrose and 18 g agar per liter of distilled water and used for regular growth of *B. maydis*. Water agar (WA) was prepared with 16 g agar per liter of distilled water and used for conidium germination. YEPD liquid medium was made from 0.3% (w/v) yeast extract, 1% peptone, 2% glucose per liter of distilled water.

2.3. Baseline sensitivity of mycelial growth of B. maydis populations to fluazinam

The baseline sensitivity of mycelial growth of *B. maydis* populations to fluazinam was determined by analyzing the distribution of 92 isolates EC_{50} (50% effective concentration) values. Briefly, mycelial plugs (5 mm diameter) from the edge of 7-day-old colony of each isolate were placed on a series of PDA plates containing 0.03125, 0.0625, 0.125, 0.25, 0.5 and 1 μg/ml fluazinam. For each concentration, three replications were conducted. Plates without fluazinam were prepared as the control. The diameters (minus the diameter of the inoculation plug) of the colonies were measured after incubation for 7 days at 25 °C in darkness. The growth inhibition as percent of control was calculated. Inhibition ratio of the fungicide on mycelial growth of each isolate in different concentrations was calculated by the formula: (the averaged diameter of control − the averaged diameter in some fungicide concentration) / (the averaged diameter of control − 5 mm of mycelial plug). The EC_{50} value for each isolate was calculated based on linear regression of colony diameter on log-transformed fungicide concentration [27]. The experiment was performed twice.

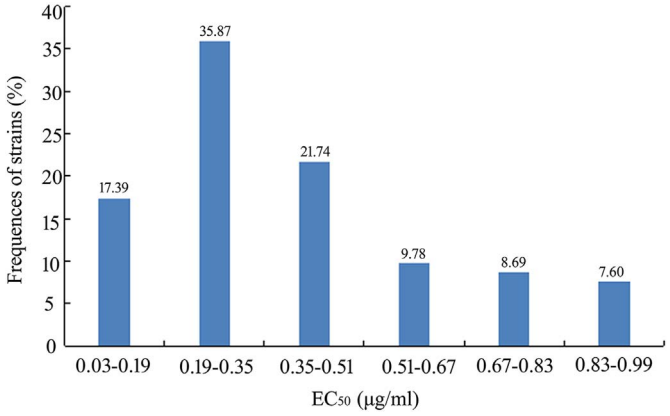

Fig. 3. Distribution of fluazinam EC_{50} values for 92 isolates of *B. maydis* by mycelial growth assay.

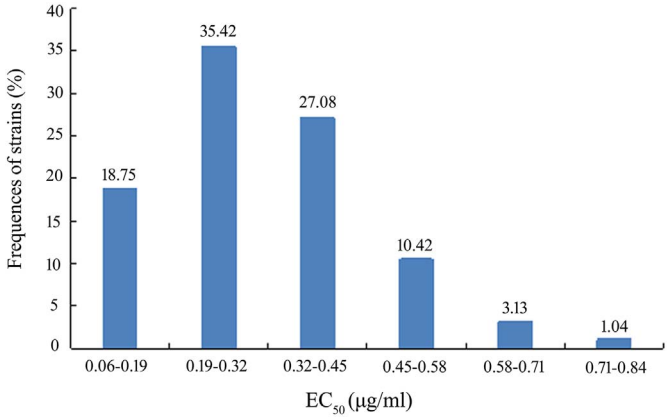

Fig. 4. Distribution of fluazinam EC_{50} values for 92 isolates of *B. maydis* by conidium germination assay.

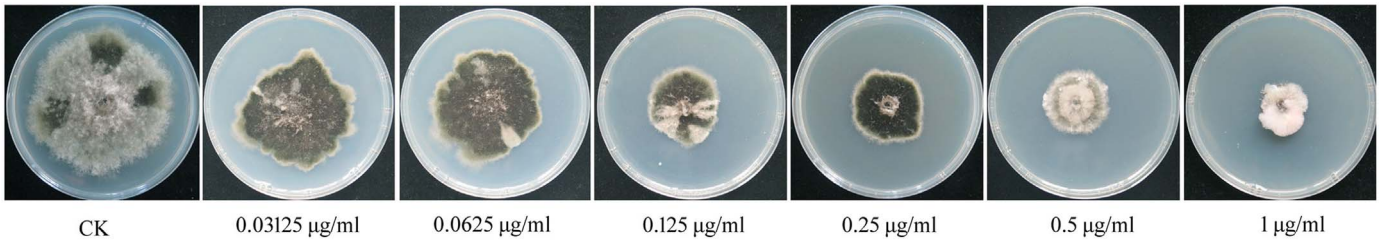

Fig. 2. The inhibition effect of fluazinam on the isolate YX43 of *B. maydis* in different concentrations of fluazinam.