Crop Protection 90 (2016) 54–58

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Activities of azoxystrobin and difenoconazole against *Alternaria alternata* and their control efficacy

Hancheng Wang [a, *], Yanfei Huang [b], Jin Wang [c], Xingjiang Chen [a], Kesu Wei [a], Maosheng Wang [a], Shenghua Shang [a]

[a] Key Laboratory of Molecular Genetics, Guizhou Academy of Tobacco Science, Guiyang 550081, PR China
[b] College of Agriculture, Yangtze University, Jingzhou 434025, PR China
[c] College of Life Science, Yangtze University, Jingzhou 434025, PR China

ARTICLE INFO

Article history:
Received 29 August 2015
Received in revised form
2 June 2016
Accepted 22 August 2016

Keywords:
Azoxystrobin
Disease control
Difenoconazole
Strobilurins

ABSTRACT

Tobacco brown spot caused by *Alteraria alternata* is a devastating disease of tobacco worldwide. In this study, we reported the effects of a strobilurin fungicide azoxystrobin and a sterol inhibitor difenoconazole on mycelial growth, spore germination, and control of brown spot. Both mycelial growth and spore germination bioassay results showed that sensitivity of *A. alternata* to difenoconazole was significantly lower than that to azoxystrobin. Azoxystrobin and the compound of azoxystrobin plus difenoconazole provided excellent control efficacy on tobacco brown spot in field. Disease control efficacies for three sprays of azoxystrobin at doses of 0.094, 0.19 and 0.28 Kg a.i./ha, of azoxystrobin plus difenoconazole at 0.15, 0.22 and 0.29 Kg a.i./ha, and of difenoconazole at 0.12 Kg a.i./ha were between 86.00% and 89.67%, between 86.14% and 89.23%, and between 55.14 and 58.41%, respectively. No phytotoxic symptoms were observed for the fungicides in field. These fungicides could potentially be used for brown spot control in tobacco.

1. Introduction

Tobacco (*Nicotiana tabacum* L.) is an annual, solanaceous plant grown commercially for its leaves. China is the biggest single tobacco market in the world, and accounts for more than 39.6% of the total global tobacco production (Wang et al., 2013) and 40% of the global tobacco consumption (Wang et al., 2014). During tobacco production period, the major destructive foliar disease tobacco brown spot occurs in every field where tobacco growing in China. This disease is caused by the notorious fungal pathogen *Alternaria alternata* (Fr.) Keissl (Main, 1969; Dobhal and Monga, 1991). It normally happens from the lower leaves of a plant and gradually spreads to the upper leaves during leaf harvest period (Stavely and Slana, 1970). The typical symptom of brown spot on leaf is a brown necrotic centre surrounded by a yellow or yellowish-green halo (Slavov et al., 2004). Tobacco leaves infected by *A. alternata* normally become incomplete, and uneven baking leaf color and leaf thickness, which results in poor quality of tobacco leaves and low value of industrial use (Jenning et al., 2002; Yakinova et al., 2009). Losses can reach more than 60% if disease management practices are not utilized. In the last five years, it has been a major serious problem for tobacco production in China (Tong et al., 2012).

The primary components of all commercial management programmes for this disease are applications of fungicides. In China, the 'traditional' fungicides used to control tobacco brown spot include dimetachlone, and the combinations of dimetachlone and protective fungicides (Zhang et al., 2009; Dong et al., 2010; Meng et al., 2013), such as dimetachlone and mancozeb. Protective fungicides normally have poor curative effect when disease has happened in the fields, and should be used in the protective manner. Due to extensive use of those fungicides for many years in tobacco commercial field in China, dimetachlone-resistant strains of *A. alternata* have occurred frequently (Li and Zhu, 2007; Luo et al., 2009; Meng et al., 2013), and the control effect of those chemicals is often poor. Recently several newly registered chemicals have been utilized extensively worldwide to control some species of *Alternaria*, including azoxystrobin (Amistar, Syngenta) (Ma et al., 2003; Rosenzweig et al., 2008; Leiminger et al., 2014; Vega and Dewdney, 2014), difenoconazole (Score, Syngenta) (Shtienberg, 1991; Vicent et al., 2007), and combinations of azoxystrobin with

* Corresponding author.
E-mail address: xiaobaiyang126@hotmail.com (H. Wang).

http://dx.doi.org/10.1016/j.cropro.2016.08.022
0261-2194/

difenoconazole (Amistar TOP, Syngenta). Up until now, few reports are involved in the sensitivities of azoxystrobin and difenoconazole on *A. alternata* isolated from host of tobacco and their efficacy for the control of tobacco brown spot in field. Due to high price of these fungicides and present regulations of the government, they have not yet been registered for tobacco brown spot control and thus have not been applied in the field in China.

The objective of this study was (i) to determine the EC_{50} and EC_{90} values of azoxystrobin and difenoconazole on mycelial growth and conidial germination of *A. alternata in vitro*; (ii) to determine their efficacy for the control of tobacco brown spot in field, and also efficacy of the treatments of azoxystrobin plus difenoconazole.

2. Materials and methods

2.1. Fungal strain, media and fungicide preparation

Isolates of *A. alternata* (C02, XY29, XY36 and XY45), with wild-type sensitivity and pathogenicity to tobacco, were collected in 2013 from infected tobacco leaves in Guizhou province of China. The isolates were grown and maintained on AEA agar (yeast powder 5 g L^{-1}, $NaNO_3$ 6 g L^{-1}, KH_2PO_4 1.5 g L^{-1}, KCl 0.5 g L^{-1}, $MgSO_4$ 0.25 g L^{-1}, glycerol 20 mL L^{-1}, and 16.0 g L^{-1} agar) (Li et al., 2005; Jin et al., 2007), in a controlled climate cabinet at 25 °C in darkness. After 7 days' incubation, conidia produced on the Petri dishes were washed with distilled water, the suspension was filtered through a double-layer of sterile cheesecloth (Grade #40: 24 × 20 threads per inch) to remove mycelial fragments and the resulting conidial suspension was diluted to a final concentration of 1×10^5 spores/ml with the help of a hemocytometer. For long-term storage, 5-mm agar plugs from the leading edge of individual colonies were transferred into several sterile 1.5-ml microcentrifuge tubes containing 1 ml of 20% sterile glycerol solution, and tubes were stored in the dark at −20 °C.

Stock fungicide solutions were prepared by dissolving technical grade azoxystrobin (93% active ingredient; Syngenta China Co., Ltd, Shanghai, China), difenoconazole (95% active ingredient; Syngenta China Co., Ltd, Shanghai, China) and salicylhydroxamic acid (SHAM) (99% active ingredient; Sigma Chemical Co., St. Louis, MO, USA) in methanol. Solutions were diluted as required and stored at 4 °C in the dark to preserve fungicide activity. The methanol concentration never exceeded 1% of the testing solution. This concentration of methanol did not affect the mycelial growth or conidial germination of *A. alternata* (data not shown). Controls always included the same methanol concentration as the test samples throughout the experiments. When the agar had cooled to approximately 50 °C, fungicides were added to the AEA agar.

Chemicals tested for efficacy against brown spot were azoxystrobin (Amistar, 250 g L^{-1} a.i., SC, Syngenta, China), difenoconazole (Score, 10% a.i., WG, Syngenta, China), and azoxystrobin plus difenoconazole (Amistar TOP, 325 g L^{-1} a.i., SC, Syngenta, China).

2.2. Inhibition of mycelial growth

Individual agar discs (5-mm-diameter) were removed from the edge of an actively growing culture and placed face up on the centre of a Petri dish (9-cm-diameter) containing AEA amended with various concentrations of test fungicides. The difenoconazole concentrations tested were chosen based on the EC_{50} value (0.78 mg L^{-1}) of difenoconazole to *A. alternata* previously described from apple (Reuveni and Sheglov, 2002). The azoxystrobin concentrations tested in combination with SHAM were chosen according to the test concentrations of azoxystrobin (0–300 mg L^{-1}) to *Alternaria solani* previously described from tomato (Zhang et al., 2008). The final concentrations tested for difenoconazole were 0, 0.16, 0.31, 0.63, 1.25, 2.50, 5 and 10 mg L^{-1}. The inhibition of azoxystrobin in combination with SHAM was tested by adding 100 mg L^{-1} SHAM to each of the concentrations, including 0, 3.13, 6.25, 12.50, 25, 50 and 100 mg L^{-1}. After incubation for 6 days at 25 °C in darkness, the untreated control reached the periphery of the plate and the diameters of the colonies for each treatment were measured. For each fungicide at each concentration, the experiments were conducted twice with three replicates.

2.3. Inhibition of conidial germination

Conidia of *A. alternata* were harvested as described above from 7-day-old cultures. Aqueous preparation of each chemical at different concentrations was added to an equal volume (250 μl) of the conidial suspension. The difenoconazole concentrations tested were chosen based on the EC_{50} value (25 mg L^{-1}) of difenoconazole to *A. alternata* previously described from apple (Reuveni and Sheglov, 2002). The azoxystrobin concentrations tested in combination with SHAM were chosen according to the test concentrations of azoxystrobin (0–3.125 mg L^{-1}) to *A. solani* previously described from tomato (Zhang et al., 2008). The control conidial suspension got an equal volume of distilled water only. The final concentrations tested for difenoconazole were 0, 3.13, 6.25, 12.5, 25, 50 and 100 mg L^{-1}. The inhibition of azoxystrobin in combination with SHAM was tested by adding 100 mg L^{-1} of SHAM to each of the concentrations, including 0, 0.025, 0.05, 0.10, 0.20 and 0.40 mg L^{-1}. Conidial suspensions were incubated at 25 °C in darkness for 12 h (Zhang et al., 2008). A conidium was regarded as germinated if the germ tube had reached at least the width of the spore. Germinated spores were quantified by counting 100 spores per site under the help of microscope. The experiments were conducted twice with three replicates for each treatment.

2.4. Field experiments

Field experiments were conducted with susceptible cultivar K326 in tobacco commercial fields in 2014 and 2015 at Guizhou Academy of Tobacco Science Research Farm in Fuquan, Guizhou province. In the field, 0.075 kg m^{-2} of a 10: 10: 25 (= N: P_2O_5: K_2O) commercial fertilizer was applied to the soil before transplanting and 0.022 kg m^{-2} of a nitrogen fertilizer (20% total nitrogen) was applied to the soil four weeks later after transplanting. In mid-May in 2014 and early-May in 2015, seedlings of tobacco, grown in 160 wells floating plates, were transplanted in the field in single rows, with 1.0 m distance between rows and 0.6 m along rows. Chemicals tested for efficacy against brown spot were listed as mentioned above. The fungicide concentrations tested were chosen according to the dosages in the product's instruction, and three dosages were selected for each fungicide. Azoxystrobin was applied at 0.094, 0.19 and 0.28 Kg a.i./ha, respectively. The mixture of azoxystrobin and difenoconazole was applied at 0.15, 0.22 and 0.29 Kg a.i./ha, respectively. Difenoconazole was sprayed at 0.12 Kg a.i./ha. Non-treated control was treated with water only throughout the experiments. For each treatment, one thousand and 50 L water per hectare was sprayed. Tobacco leaves were sprayed from both sides until runoff with a sprayer (Redsun 3WBD-16, Tongzhou Pingchao Yangguang Proctection Factory, Jiangsu, China). When around 1% leaves presented brown spot symptom, these fungicides were applied for the first time. In 2014, sprays were applied on 24th July, and 4th and 12th August. In 2015, sprays were applied on 14th and 24th July, and 3rd August. On 3rd and 20th August in 2014, and 25th July and 11th August in 2015 (ten days after the second and the third spray, respectively), severity scale of each leaf was investigated based on a 0 to 9 scales: 0, no visible disease symptoms; 1, few symptoms (less than 1% leaf areas); 3, few symptoms (between