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Evaluation of the combination of propamocarb hydrochloride and fluopicolide for management of black shank on tobacco

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ABSTRACT

Phytophthora nicotianae, the causal agent of black shank, is responsible for huge yield losses and quality reduction in global tobacco production. Effective use of fungicides continues to be the major component in developing integrated disease management (IDM) programme. Infinito is an oomycete-specific fungicide that developed by Bayer CropScience, with a novel mode of action (active substances: propamocarb hydrochloride and fluopicolide). So far, limited information is available regarding the black shank control efficacy of Infinito in laboratory, greenhouse and field trials. Experiments were performed and our results showed that propamocarb hydrochloride, fluopicolide and their combination inhibited mycelial growth of *P. nicotianae* with EC_{50} values of 3.30, 0.20 and 0.06 mg L^{-1}, respectively. In the greenhouse, Infinito reduced the disease incidence from 86.7% to 21.0% compared with untreated group. Besides the excellent ability to control black shank, Infinito also had a stimulating effect on tobacco seedlings growth. Similar results of field trials revealed that Infinito could not only reduce tobacco black shank incidence but also increase tobacco height, leaf area and yield. Consequently, the results indicate that Infinito is a new suitable fungicide for controlling tobacco black shank, offering a promising component in developing integrated programs for effective management of the disease.

1. Introduction

Tobacco is one of the most important industrial and economic crops in the world. China dominates the global total yield of tobacco, exceeding one-third of the world's total with about 16.2 million hectares planting area every year. In addition to be the major foreign exchange earner, tobacco were also planted in China as a medicinal plants by providing potential new sources of cancer-preventive extracts (Baxter et al., 2017). However, tobacco production is mostly hampered by plant diseases, of which the most vicious one is black shank (Jing et al., 2017). This disease occurs annually in the major tobacco production areas in China, causing serious losses in yield and affecting the tobacco quality (Wang et al., 2013).

Tobacco black shank, caused by oomycete pathogen *Phytophthora nicotianae* (syn. *P. parasitica* var. *nicotianae*), was firstly discovered on java island of Indonesia and named by van Breda de Haan in 1896 (Speight, 1994; Zhang et al., 2003). The disease is characterized by a rapid yellowing and wilting followed by death of the entire plant. Then the plant piths becoming plate-like that turn black or brown (Csinos, 1999). Infected tobacco roots may be decayed and plants may die eventually when severely occurred (Parkunan et al., 2010). Previous studies have shown that black shank has four physiological races (race 0, 1, 2 and 3), with race 0 and 1 being the most prevalent races in China (Gallup and Shew, 2010; Li et al., 2017).

Tobacco black shank is among the most stubborn diseases, due to long-term survival of the pathogen in the soil and attacking all growing stages of the tobacco plants. Crop rotation has limited value and is not usually adopted by growers. Breeding of disease resistant varieties was the most economic and effective measure previously (Ji et al., 2014), however, the pathogen population evolve rapidly in the field and accelerate the development of new pathogen races, makes the cultivars resistant ineffective in disease control in recent years (Nifong et al., 2011; McCorkle et al., 2013). Consequently, the application of fungicides is the primary approach in managing tobacco black shank (Antonopoulos et al., 2010). In China, products containing metalaxyl and dimethomorph have been considered to be the most widely used fungicides to control black shank in the past decades (Yuan et al., 2006; Yang et al., 2015). However, field isolates of *Phytophthora* spp. had

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diverse degrees in insensitivity to metalaxyl and dimethomorph (Parra and Ristaino, 2001; Hu et al., 2008). Therefore, screening novel and environmental friendly fungicide is desirable for enhancing the control efficacy of tobacco black shank and reducing selection pressure for fungicide resistance development.

The formula of Infinito suspension concentrate (SC) is developed by Bayer Crop Science, combines the activity of fluopicolide and propamocab hydrochloride. Fluopicolide is benzamide class of fungicides which is significantly effective against oomycetes, can inhibit the growth of black shank mycelium and suppress the production of *P. nicotianae* sporangia (Wang et al., 2014; Qu et al., 2016a,b). Propamocarb hydrochloride, as a systemic fungicide, is commonly used for the control of *Phytophthora* diseases (Cohen and Coffey, 1986). So far, few studies have examined the control effect of Infinito on tobacco black shank. The main objective of the present study was to evaluate the efficacy of the combination of fluopicolide and propamocab hydrochloride for disease control in greenhouse and field trials. These results may offer new approaches for control of tobacco black shank.

2. Material and methods

2.1. Fungicides

Metalaxyl (purity 95.0%) and its commercial formulation (Ridomil, 25% Wettable Powder, WP) were obtained from Rainbow Chemical Co., Ltd, Weifang, China. Mefenoxam (purity 91%), propamocarb hydrochloride (purity 97%), fluopicolide (purity 98%), dimethomorph (purity 97%) and its commercial formulation (Acrobat, 50% WP) were obtained from Shandong Sino-Agri Union Biotechnology Co., Ltd, Tai'an, China. The ratio of the combination of fluopicolide and propamocarb hydrochloride active ingredient (a.i.) was 1:10, and the combination commercial formulation (Infinito SC, fluopicolide 62.5 g a. i. L^{-1} plus propamocab hydrochloride 625 g a. i. L^{-1}) was obtained from Bayer Crop Science Co., Ltd, Hangzhou, China.

2.2. Isolation and identification of P. nicotianae

Isolation and identification of *P. nicotianae* was conducted as previously described by Forbes et al. (1997). *P. nicotianae* was cultured for 7 day at 27 ± 1 °C in darkness on an oat medium (OA) plate. Preparation method of OA was as follows: oatmeal 45 g with 1000 mL water are heated on a boiling water bath for 1 h, filtered with gauze, and water was added to a total volume of 1000 mL. Then 20 g agar was added and sterilized (121 °C, 30 min).

2.3. Tobacco seedlings

The soil nutrition matrix (perlite: vermiculite: peat = 1: 3: 1) was moistened with distilled water, and autoclaved for 45 min at 121 °C on two consecutive days. Tobacco seeds (cv. NC55) were sown in seedling trays (3.6 × 3.6 × 5.0 cm) with soil nutrition matrix.

2.4. Inhibition of mycelial growth of P. nicotianae by fungicides

P. nicotianae growth rate was measured on the OA media amended with different fungicides. All fungicide solutions were dissolved in acetone and then diluted into a series of concentrations with distilled water containing 0.1% Tween 80. Metalaxyl (1.00, 5.00, 10.00, 20.00, 50.00 mg L^{-1}), mefenoxam (0.125, 0.25, 0.50, 1.00, 2.00 mg L^{-1}), dimethomorph (0.25, 0.50, 1.00, 2.00, 5.00 mg L^{-1}), fluopicolide (0.05, 0.10, 0.20, 0.40, 0.80 mg L^{-1}), propamocarb hydrochloride (1.00, 2.00, 4.00, 8.00, 10.00 mg L^{-1}), combination of fluopicolide and propamocarb hydrochloride (0.01, 0.02, 0.05, 0.10, 0.20 mg L^{-1}) were prepared. OA plates amended with 1 mL of series of diluted fungicides were prepared. OA plates amended with 1 mL of sterile distilled water were used as controls. All plates were inoculated with a five-mm-diameter agar plug excised from the actively growing front of seven-days-old colonies of the *P. nicotianae*. All actions were conducted in a laminar flow cabinet. Inoculated plates were incubated for 5 day at 27 ± 1 °C in darkness. After five days of incubation, colony diameters (minus the plug diameter) were measured in two perpendicular directions using a measuring ruler (Ferrin and Kabashima, 1991). The inhibitory effect of each fungicide concentration was evaluated with four replications. Inhibition of mycelial linear growth was calculated according to the equation: inhibition of mycelial linear growth (%) = [1-(diameter of treated-diameter of fungal disk)/(diameter of control-diameter of fungal disk)] · 100. The relative diameter of *P. nicotianae* on control plates was used to determine the EC_{50} and EC_{90} value of the fungicide. This experiment was performed at three independent times.

2.5. Control effects of fungicides on tobacco black shank in greenhouse

A pot-trial experiment was conducted under greenhouse conditions with temperatures ranging from 25 to 28 °C in Shandong Agricultural University. Tobacco seeds (cv. NC55) without seed coating treatment were used. Tobacco seeds were planted in a tray and chosen when they had the sixth true leaves (Qu et al., 2016a,b). Tobacco seedlings of uniform growth were selected and transplanted to the pots containing sterilized potting soil (20 × 20 × 15 cm) in a greenhouse. One week after that, the seedlings were treated with Infinito (386.7 g a. i. ha^{-1} or 773.4 g a. i. ha^{-1}), metalaxyl (375 g a. i. ha^{-1}), and dimethomorph (300 g a. i. ha^{-1}), and distilled water was used as an untreated control. Treatments were applied as sprays until the liquid along the tobacco plant stem flow into the rhizosphere around the topsoil (50 mL per pot). The treated pots were inoculated with *P. nicotianae* one week later, 100 mL spore suspensions (10^6 spores mL^{-1}) were applied on the root of each tobacco plant. The *P. nicotianae* spore suspensions were obtained by inoculating one mycelial disc (0.5 cm diameter) of the previous culture to an Erlenmeyer flask with 200 mL OA medium and incubated for 21 days at 25 °C shaker (Davison, 1998; Hu et al., 2007). Each treatment was represented by four replicates with 20 pots per replicate. Pathogen-inoculated pots were randomly arranged and watered as needed. The tobacco plant height, stem diameter, effective leaves, photosynthetic efficiency number and disease incidence were recorded on the 21st day after pathogen inoculation. Pathogenicity was evaluated by assessing the degree of wither in the tobacco leaves. Black shank severity was rated on a standard scale from 0 to 9 (Han et al., 2016): 0 = no symptoms; 1 = less than one third of the total leaves are wilted; 3 = one third to a half of the total leaves are wilted; 5 = one half to two thirds of the total leaves are wilted; 7 = more than two thirds of total leaves are wilted; 9 = plant was dead. The disease incidence, disease index and disease control effects were calculated using the following formulas:

Disease incidence (%) = (total number of diseased seedlings/total number of emerged seedlings) × 100

Disease index = Σ [(rating × number of plants rated)/(total number of plants × highest rating)] × 100

Disease control effect (%) = [(disease incidence of the control − disease incidence of the treatment)/disease incidence of the control] × 100

2.6. Control effects of fungicides against tobacco black shank in field studies

In May 2017, field trials were conducted in commercial tobacco fields at Weifang and Tai'an, Shandong Province, China. The farmlands had been in conventional tobacco production for decade years, and had a history of heavy black shank on tobacco in previous years. The soil types were both sandy clay with organic matter content 12.7 g kg^{-1} and 10.2 g kg^{-1} in the tillage layer (0–20 cm) at Weifang and Tai'an,