

# Field Prevention and Control of Tobacco Black Shank in Guizhou Province

Yingjie JIANG<sup>1</sup>, Yongmo LI<sup>2</sup>, Jun ZHOU<sup>1</sup>, Laifu LUO<sup>1</sup>, Yinsheng WANG<sup>2</sup>

1. Syngenta (China) Investment Co., Ltd., Zhengzhou 450008, China; 2. Zunyi Shijia Pesticide Trading Co., Ltd., Zunyi 563099, China

**Abstract** [ **Objectives** ] The paper was to explore the prevention and therapeutic schedule of tobacco black shank. [ **Methods** ] Different concentrations of 25 g/L fludioxonil · 37.5 g/L metalaxyl-M, 10 billion/mL *Bacillus amyloliquefaciens*, 110 g/L amino acids · 24 g/L manganese zinc, 120 g/L calcium · 20 g/L magnesium, and 4% metalaxyl-M · 64% mancozeb were employed to assess their efficacy in controlling tobacco black shank. The disease index was subsequently evaluated. [ **Results** ] 25 g/L fludioxonil · 37.5 g/L metalaxyl-M + 10 billion/mL *B. amyloliquefaciens* + 110 g/L amino acids · 24 g/L manganese zinc (transplanting dosage) or 120 g/L calcium · 20 g/L magnesium (sealing dosage) (transplanting dosage:  $750 \text{ mL/hm}^2 + 1.2 \times 10^4 \text{ mL/hm}^2 + 1.5 \times 10^3 \text{ mL/hm}^2$ , sealing dosage:  $1.5 \times 10^3 \text{ mL/hm}^2 + 1.2 \times 10^4 \text{ mL/hm}^2 + 1.5 \times 10^3 \text{ mL/hm}^2$ ) resulted in a notable impact on the prevention of tobacco black shank. The incidence in the treated area was 10.78%, a 35.72% reduction compared to the control. The estimated yield was 99 700 yuan/hm<sup>2</sup>, a 34.91% increase compared to the control. 25 g/L fludioxonil · 37.5 g/L metalaxyl-M + 10 billion/mL *B. amyloliquefaciens* + 120 g/L calcium · 20 g/L magnesium + 4% metalaxyl-M · 64% mancozeb (control dosage:  $1.5 \times 10^3 \text{ mL/hm}^2 + 1.2 \times 10^4 \text{ mL/hm}^2 + 1.5 \times 10^3 \text{ mL/hm}^2 + 1.5 \times 10^3 \text{ g/hm}^2$ ,  $1.5 \times 10^3 \text{ mL/hm}^2 + 1.2 \times 10^4 \text{ mL/hm}^2 + 1.5 \times 10^3 \text{ mL/hm}^2 + 1.5 \times 10^3 \text{ g/hm}^2$ , with an interval of 7 d between applications) demonstrated a significant efficacy in controlling tobacco black shank. At 7 d following the second application, the relative preventive efficacy was observed to be 88.99%. Additionally, the estimated yield was 109 900 yuan/hm<sup>2</sup>, representing an increase of 244.51% compared to the control. [ **Conclusions** ] During the transplanting and sealing stages, 25 g/L fludioxonil · 37.5 g/L metalaxyl-M + 10 billion/mL *B. amyloliquefaciens* + 110 g/L amino acids · 24 g/L manganese zinc (transplanting dosage) or 120 g/L calcium · 20 g/L magnesium (sealing dosage) may be employed to enhance the growth of tobacco plants and mitigate the occurrence of tobacco black shank. Additionally, 25 g/L fludioxonil · 37.5 g/L metalaxyl-M + 10 billion/mL *B. amyloliquefaciens* + 120 g/L calcium · 20 g/L magnesium + 4% metalaxyl-M · 64% mancozeb can be utilized for the treatment of tobacco black shank during the initial incidence stage.

**Key words** Tobacco black shank; Prevention; Treatment; Field

## 1 Introduction

Tobacco is classified within the genus *Nicotiana*, family Solanaceae, order Tubiflorae, class Dicotyledoneae, and division Angiospermae. Approximately 70 varieties of tobacco are recognized; however, only two varieties, *Nicotiana tabacum* and *Nicotiana rustica*, are commonly cultivated and utilized for smoking purposes. Currently, the most prevalent types of tobacco, including flue-cured tobacco, dried tobacco, white-ribbed tobacco, cigar tobacco, and spice tobacco, are primarily derived from *N. tabacum*. In contrast, *N. rustica* is predominantly utilized in the production of pipe tobacco and hookah. In the beginning, humans chewed tobacco leaves as a means of preventing illness and banishing malevolent spirits. It was only later that this practice evolved into the act of smoking.

As a major cash crop in China, tobacco generates considerable economic value for the country on an annual basis. Tobacco was introduced during the Ming Dynasty primarily for its medicinal properties and experienced rapid proliferation in the subsequent Qing Dynasty. The earliest recorded smoking ban was enacted in 1639<sup>[1]</sup>, while the *World Health Assembly* was established in 2003. By the year 2021, tobacco tax revenue had reached 1 358.1 billion yuan<sup>[2]</sup>. In 2021, the total area dedicated to tobacco cultivation in China was 1 012.92 thousand hm<sup>2</sup>. Within this context, Guizhou province, with an area of 136.8 thousand hm<sup>2</sup>, ranked second in

terms of tobacco production.

Tobacco black shank is a significant soil-borne fungal disease affecting tobacco plants, caused by *Phytophthora parasitica* var. *nicotianae* (Breda de Haan) Tucker. This disease is recognized as one of the most destructive threats to tobacco production. The first discovery of tobacco black shank was made in Java by Bred de Haan in 1896. The disease was initially identified in China between 1919 and 1945 in Sawada Kanekichi's report on the survey of mycorrhizal fungi produced in Taiwan, China. Its occurrence in Shandong Province is estimated to have occurred between 1927 and 1928. In addition to Heilongjiang Province, where no occurrences have been reported, the remaining tobacco-growing provinces and regions in China, including Taiwan, have experienced incidents. Provinces such as Henan, Shandong, Anhui, Zhejiang, Hunan, Hubei, Fujian, Guangdong, Guangxi, Yunnan, Guizhou, and Sichuan have reported more frequent occurrences, with the damage being more severe. The disease may manifest at any point from the resettling stage to the maturity stage of tobacco. The primary symptoms include a sudden wilting of the leaves, which subsequently turn yellow and wilt within a few days. As the disease progresses, a significant portion or the entirety of the root system and the base of the stem may become blackened and decayed, ultimately leading to the death of the entire plant. A longitudinal examination of the affected stems indicates that the pith undergoes desiccation and contraction, resulting in the formation of brownish discs interspersed with sparse white mycelium. This characteristic is the primary distinguishing feature of tobacco black

shank in comparison to other root and stem diseases<sup>[3]</sup>.

The emergence of tobacco black shank is often exacerbated by rainfall, leading to widespread devastation. The mild consequences include a reduction in yield, while severe cases may result in the cessation of harvest activities, thereby causing significant economic losses for tobacco farmers. Currently, the prevention and treatment of tobacco black shank predominantly rely on the use of individual agents. For instance, a suspension of 23.4% mandipropamid demonstrates a disease control efficacy of 80.11% against tobacco black shank<sup>[4]</sup>. Additionally, the combination of metalaxyl-M and fludioxonil has shown effective control over tobacco black shank<sup>[5]</sup>. Furthermore, a concentration of 10 billion/mL *Bacillus amyloliquefaciens* WP exhibits a control efficacy of 63.1% against tobacco black shank<sup>[6]</sup>. Furthermore, 25 g/L fludioxonil · 37.5 g/L metalaxyl-M functions as a fungicide with an extensive bactericidal spectrum. It demonstrates a significant efficacy in controlling various pathogens, including *Phytophthora*, *Achlya prolifera*, *Pythium oryzae*, *Fusarium moniliforme*, *Fusarium graminearum*, *Fusarium oxysporum*, *Rhizoctonia solani*, and *Drechslera oryzae*. 10 billion/mL *B. amyloliquefaciens* functions as a bactericide, demonstrating efficacy in enhancing soil properties and promoting root development. In Guizhou, the rainy climate that prevails from April to June each year creates favorable conditions for the proliferation of tobacco black shank. This phenomenon leads to extensive damage across the region, significantly reducing the yield of tobacco plants or even resulting in their complete loss. Consequently, this situation incurs substantial economic losses. This study was conducted to develop an improved combination control program for tobacco black shank, with the ultimate objective of enhancing tobacco plant production and increasing the

income of tobacco farmers.

2 Materials and methods

**2.1 Materials** The test was conducted in Jindao Village, Yanxi Town, Bozhou District, Zunyi City, Guizhou Province, in the year 2023, utilizing Yunyan 87 as the test subject. The agents utilized in the experiment comprised Liangdun (25 g/L fludioxonil · 37.5 g/L metalaxyl-M, Syngenta), Dijian (10 billion/mL *B. amyloliquefaciens*, Syngenta), Ruikeming (110 g/L amino acids · 24 g/L manganese zinc, Syngenta), Yishi Qiushi (120 g/L calcium · 20 g/L magnesium, Syngenta), and Jinlei (4% metalaxyl-M · 64% mancozeb, Syngenta).

2.2 Methods

**2.2.1 Survey method and test scheme.** The growth of tobacco plants was examined by selecting five sampling points for each treatment, with five plants assessed at each point. Data were collected on the number of leaves per plant, as well as the length and width of the top six to eight leaves. The investigation concentrated on tobacco black shank, with a particular emphasis on evaluating the disease index to determine the incidence of the disease among all tobacco plants within the experimental area. The severity of the disease can be quantified according to a standard grading system. Grade 0 indicates the absence of disease on the entire plant. Grade 1 is characterized by the withering of less than 1/3 of the leaves. Grade 3 represents the withering of 1/3 to 1/2 of the leaves, or the appearance of spots on a few lower leaves. Grade 5 denotes the withering of 1/2 to 2/3 of the leaves. Grade 7 indicates the withering of more than 2/3 of the leaves. Grade 9 signifies the death of the plant due to the disease. The fundamental details of the testing scheme are presented in Table 1.

Table 1 Fundamental details of the testing scheme

Test	Treatment	Agent and dosage	Application period	Water consumption
1	Treatment I	750 mL/hm <sup>2</sup> Liangdun (25 g/L fludioxonil · 37.5 g/L metalaxyl-M) + 1.2 × 10 <sup>4</sup> mL/hm <sup>2</sup> Dijian (10 billion/mL <i>Bacillus amyloliquefaciens</i> ) + 1.5 × 10 <sup>3</sup> mL/hm <sup>2</sup> Ruikeming (110 g/L amino acids · 24 g/L manganese zinc)	April 16, 2023	Root-irrigation, 4 500 L/hm <sup>2</sup>
		1.5 × 10 <sup>3</sup> mL/hm <sup>2</sup> Liangdun (25 g/L fludioxonil · 37.5 g/L metalaxyl-M) + 1.2 × 10 <sup>4</sup> mL/hm <sup>2</sup> Dijian (10 billion/mL <i>B. amyloliquefaciens</i> ) + 1.5 × 10 <sup>3</sup> mL/hm <sup>2</sup> Yishi Qiushi (120 g/L calcium · 20 g/L magnesium)	May 14, 2023	Root-irrigation, 4 500 L/hm <sup>2</sup>
		Control Blank control	—	—
2	Treatment II	1.5 × 10 <sup>3</sup> mL/hm <sup>2</sup> Liangdun (25 g/L fludioxonil · 37.5 g/L metalaxyl-M) + 1.2 × 10 <sup>4</sup> mL/hm <sup>2</sup> Dijian (10 billion/mL <i>B. amyloliquefaciens</i> ) + 1.5 × 10 <sup>3</sup> mL/hm <sup>2</sup> Yishi Qiushi (120 g/L calcium · 20 g/L magnesium) + 1 500 g/hm <sup>2</sup> Jinlei (4% metalaxyl-M · 64% mancozeb)	Early onset of tobacco black shank, June 25, 2023	Root-irrigation, 7 500 L/hm <sup>2</sup>
		1.5 × 10 <sup>3</sup> mL/hm <sup>2</sup> Liangdun (25 g/L fludioxonil · 37.5 g/L metalaxyl-M) + 1.2 × 10 <sup>4</sup> mL/hm <sup>2</sup> Dijian (10 billion/mL <i>B. amyloliquefaciens</i> ) + 1.5 × 10 <sup>3</sup> mL/hm <sup>2</sup> Yishi Qiushi (120 g/L calcium · 20 g/L magnesium) + 1 500 g/hm <sup>2</sup> Jinlei (4% metalaxyl-M · 64% mancozeb)	7 d following the first application, June 1, 2023	Root-irrigation, 7 500 L/hm <sup>2</sup>
		Control Blank control	—	—

NOTE The water consumption is 15 000 plants/hm<sup>2</sup>.

2.2.2 Calculation methods.

Dead seedling rate (%) = Number of dead seedlings/Total number of seedlings surveyed × 100%

Disease index = ∑ (Number of diseased plants at each grade × Representative value of the grade)/(Total number of surveyed plants

(1)

$$\times \text{Representative value of the highest grade}) \times 100$$
$$\text{Relative control efficiency} = (\text{Disease index in the control group} - \text{Disease index in the treatment group}) / \text{Disease index in the control group} \times 100$$
$$\text{Output value per leaf} = \text{Total output value} / \text{Total number of leaves}$$
$$\text{Output value per plant} = \text{Output value per leaf} \times \text{Number of leaves per plant}$$
$$\text{Expected yield per hm}^2 = 1\,000 \times \text{Harvesting rate} \times \text{Output value per plant}$$

The calculation was predicated on a density of 15 000 tobacco plants per hm<sup>2</sup>.

(2)

(3)

(4)

(5)

(6)

3 Results and analysis

**3.1 Test 1** The study demonstrated that treatment I had a significant preventive effect on the plots affected by tobacco black shank, resulting in an incidence of 10.78%. This represents a reduction of 35.72% compared to the control (Table 2). Furthermore, this program demonstrated significant advantages for the growth of tobacco plants. The number of leaves per plant increased to 16.28, representing a 5.58% enhancement compared to the control. Additionally, the plant height reached 108.00 cm, indicating a 36.48% increase relative to the control. The average leaf length was observed to be 81.06 cm, representing a 28.36% increase compared to the control, while the average leaf width reached 30.20 cm, demonstrating a 27.32% increase compared

to the control (Table 3). The final harvesting rate was 92.8%, representing an increase of 10.36% compared to the control (Table 4). The quality of the tobacco produced was significantly improved in comparison to the blank control. For instance, the output value of C2F tobacco leaves reached 1 913.59 yuan, representing 55.52% of the total output value, and was 229.69% higher than that of the control, which recorded an output value of 16.84% (Table 5). The output value of each leaf reached 0.44 yuan/leaf, representing a 15.79% increase over the blank control. The output value of each plant reached 7.16 yuan/plant, indicating a 22.18% increase over the control. The estimated yield was 99 700 yuan/hm<sup>2</sup>, reflecting a 34.91% increase over the control (Table 6).

Table 2 Incidence of tobacco black shank (June 1, 2023)

Treatment	Total number of plants	Number of diseased plants	Diseased plant rate//%	Decrease rate//%
Treatment I	816	88	10.78	35.72
Control	465	78	16.77	–

Table 3 Growth of tobacco plants (July 2, 2023)

Treatment	Number of leaves	Increase rate//%	Plant height//cm	Increase rate//%	Average leaf length//cm	Increase rate//%	Average leaf width//cm	Increase rate//%
Treatment I	16.28	5.58	108.00	36.48	81.06	28.36	30.20	27.32
Control	15.42	–	79.13	–	63.15	–	23.72	–

Table 4 Fundamental details of harvesting

Treatment	Total number of plants	Number of harvesting plants	Harvesting rate//%	Increase rate//%
Treatment I	816	758	92.80	10.36
Control	465	391	84.09	–

**NOTE** The occurrence of tobacco black shank in this experimental area resulted in a reduced harvesting rate.

Table 5 Yield information

Grade and price (including subsidies)	Treatment I			Control		
	Number//piece	Weight//kg	Output value//Yuan	Number//piece	Weight//kg	Output value//yuan
B2F//33.5 yuan/kg	521	9.24	309.54	221	3.10	103.85
B3F//24.2 yuan/kg	451	7.63	184.65	1 155	16.62	402.20
C2F//42.6 yuan/kg	3 536	44.92	1 913.59	513	5.40	230.04
C3F//38 yuan/kg	1 855	19.98	759.24	1 351	15.31	581.78
C3F//26.8 yuan/kg	944	10.43	279.52	227	1.80	48.24
Abandoned	471	5.28	0	142	1.59	0
Total	7 778.00	97.48	3 446.54	3 609	43.82	1 366.11

Table 6 Estimated output

Treatment	Output value per leaf//yuan/leaf	Number of leaves per plant//leaf/plant	Output value per plant//yuan/plant	Estimated output//10 <sup>4</sup> yuan/hm <sup>2</sup>
Treatment I	0.44	16.28	7.16	9.97
Control	0.38	15.42	5.86	7.39

**3.2 Test 2** This test demonstrated that treatment II was highly effective in managing tobacco black shank, exhibiting a relative efficacy of 96.41% 7 d following the initial application (*i. e.*, prior to the second application) and 88.99% 7 d following the second application (Table 7). The final harvesting rate was recorded at 94.15%, representing an increase of 62.90% compared to the blank control (Table 8). The quality of the tobacco produced was significantly improved in comparison to the blank control. Specifically, the output value of the C2F tobacco leaves reached 565.30

yuan, representing 61.48% of the total output value. This figure reflected an increase of 39.06% over the output value of the blank control, which was 44.21% (Table 9). The output value per leaf was determined to be 0.48 yuan, reflecting an increase of 17.07% compared to the blank control. Additionally, the output value per plant was calculated at 7.78 yuan, representing a 66.60% increase over the blank control. Furthermore, the estimated output was 109 900 yuan per hm<sup>2</sup>, indicating a substantial increase of 244.51% relative to the blank control (Table 10).

Table 7 Control effect of tobacco black shank

Survey period	Treatment	Total number of plants	Number of diseased plants	Diseased plant rate//%	Disease index	Relative control efficiency//%
Prior to the first application, May 25, 2023	Treatment II	171	25	14.62	4.35	–
	Control	134	16	11.94	3.48	–
Prior to the second application, June 1, 2023	Treatment II	171	12	7.02	0.84	96.41
	Control	134	24	40.30	23.38	–
7 d following the second application, June 8, 2023	Treatment II	171	12	7.02	4.42	88.99
	Control	134	80	59.70	40.13	

Table 8 Fundamental details of harvesting

Treatment	Total number of plants	Number of harvesting plants	Harvesting rate//%	Increase rate//%
Treatment II	171	161	94.15	62.90
Control	134	61	45.52	–

Table 9 Yield information

Grade and price (including subsidies)	Treatment II			Control		
	Number//piece	Weight//kg	Output value//Yuan	Number//piece	Weight//kg	Output value//yuan
B2F//33.5 yuan/kg	171	3.59	120.27	0	0	0
B3F//24.2 yuan/kg	392	3.62	87.60	132	1.45	35.09
C2F//42.6 yuan/kg	993	13.27	565.30	159	2.25	95.85
C3F//38 yuan/kg	367	3.85	146.30	195	2.26	85.88
C3F//26.8 yuan/kg	0	0	0	0	0	0
Abandoned	0	0	0	37	0.37	0
Total	1 923	24.33	919.47	523	6.33	216.82

Table 10 Estimated output

Treatment	Output value per leaf//yuan/leaf	Number of leaves per plant//leaf/plant	Output value per plant//yuan/plant	Estimated output//10 <sup>4</sup> yuan/hm <sup>2</sup>
Treatment II	0.48	16.2	7.78	10.99
Control	0.41	11.4	4.67	3.19

## 4 Conclusions and discussion

Following the completion of the study, it was recommended to utilize program 1, which consists of 25 g/L fludioxonil · 37.5 g/L metalaxyl-M at a rate of 750 mL/hm<sup>2</sup> for transplanting, or 1 500 mL/hm<sup>2</sup> for sealing, in conjunction with 10 billion/mL of *B. amyloliquefaciens* at a rate of 1.2 × 10<sup>4</sup> mL/hm<sup>2</sup>, and 110 g/L amino acids · 24 g/L manganese zinc (transplanting dosage) or 120 g/L calcium · 20 g/L magnesium (sealing dosage) at a rate of 1 500 mL/hm<sup>2</sup>, for root irrigation. This approach necessitates a water consumption of 4 500 L/hm<sup>2</sup>. It is important to note that dry weather conditions may lead to increased water consumption, which can subsequently facilitate the revitalization of seedlings and the growth of tobacco plants, thereby achieving the desired effect of preventing tobacco black shank.

Furthermore, it is important to note that preventive measures do not provide an absolute assurance against the recurrence of tobacco black shank. If tobacco black shank manifests in neighboring plots, the bacteria may also disseminate to the treated area via rainwater transmission. Therefore, the application of treatment II, which consists of 25 g/L fludioxonil · 37.5 g/L metalaxyl-M at a rate of 1 500 mL/hm<sup>2</sup>, 10 billion/mL *B. amyloliquefaciens* at a rate of 1.2 × 10<sup>4</sup> mL/hm<sup>2</sup>, 120 g/L calcium · 20 g/L magnesium at a rate of 1 500 mL/hm<sup>2</sup>, and 4% metalaxyl-M · 64% mancozeb at a rate of 1 500 g/hm<sup>2</sup>, is recommended for the prevention of tobacco black shank. It is advisable to implement this program during the early stages of the disease, specifically at the onset of sudden wilting and drooping of the apical leaves. A second appli-

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menting disinfestation procedures. Secondly, while some nurseries directly purchase substrates, not all manufacturers ensure that the substrates are disinfested prior to distribution. This gap in the disinfestation process warrants further attention.

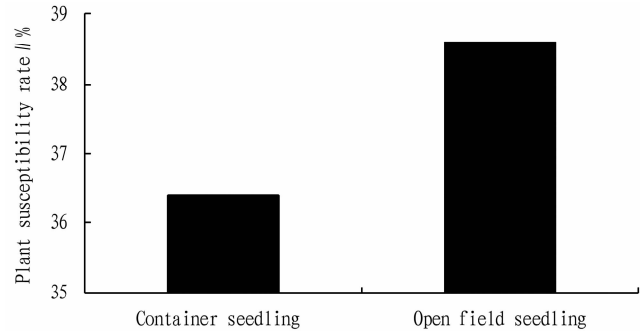


Fig.2 Results of nematode plant susceptibility in citrus nurseries

4 Conclusions

A survey conducted on citrus nurseries revealed that the detection rate of HLB in virus-free nurseries, both inside and outside the province, was found to be zero. Given the heightened awareness of HLB and the enhancement of comprehensive prevention and control measures in China, virus-free nurseries that propagate seedlings have prioritized the prevention and control of HLB. In this context, the management of pathogen sources within the nurseries has proven to be a critical factor in the effective control of HLB. Currently, the establishment of virus-free citrus nurseries in Guangdong Province is increasingly adhering to standardized practices. Each nursery has developed its own detoxified parental gardens, cutting orchards, and seedling nurseries. However, testing conducted within these nurseries has revealed certain issues that require attention. The recent introduction of new citrus varieties, including ‘Wogan’, ‘Jinqiushatangju’, and ‘Cuimijinju’ has resulted in a rapid expansion of the citrus market. However, this growth has created a significant supply gap for high-quality, virus-free citrus seedlings. In pursuit of economic gains, seedling producers have compromised their risk management practices concerning diseases and pests to expedite seedling production. Consequently, the efforts to maintain virus-free maternal stock have

lagged, and the sources of scions have not been adequately purified. This situation has contributed to the spread of specific quarantine diseases and other detrimental pathogens within the citrus industry. Nevertheless, this issue has not received sufficient attention from nursery enterprises.

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cation should be administered 7 d after the initial application to effectively manage the disease. Typically, tobacco black shank manifests during the resettling stage, when the root system is more developed. To optimize the control efficacy, it is essential to increase water application to 7 500 L/hm<sup>2</sup>, thereby enhancing the overall treatment effectiveness.

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