Survey and Detection of Disease and Pest Occurrence in Citrus Nurseries

Liying GUO[1,2], Qinghao SONG[2], Ying HE[2], Jiayi TANG[2], Xiaoting ZHUANG[2], Guochan HUANG[3], Qianhua JI[1,2]*, Yaping HU[2]

1. Institute of Pomology, Zhaoqing University, Zhaoqing 526361, China; 2. College of Life Sciences, Zhaoqing University, Zhaoqing 526361, China; 3. Sihui Science and Technology Demonstration Park, Zhaoqing 526061, China

Abstract [**Objectives**] The paper was to ascertain the prevalence of diseases and pests in a range of citrus nurseries situated in Guangdong Province and its neighboring provinces. [**Methods**] Citrus diseases and pests were systematically investigated, and citrus leaf samples were randomly collected from 15 citrus nurseries across 8 regions in Guangdong Province and its neighboring provinces. Quantitative polymerase chain reaction (qPCR) and reverse transcription polymerase chain reaction (RT-PCR) techniques were employed to detect diseases in the collected samples. Additionally, root and substrate samples were obtained, and root-knot nematodes were isolated using the Baermann funnel method. [**Results**] The positive detection rate of citrus huanglongbing (HLB) was recorded at 3%, indicating an increase in attention towards this disease compared to 2013. Additionally, the positive detection rate for citrus bacterial canker disease (CBCD) was found to be 16.5%. It was observed that the majority of nurseries with positive samples employed open field rearing practices without the use of mesh chambers, and the primary source of scions was self-propagation. The detection rate of citrus tristeza virus (CTV) was found to be the highest, with a positive detection rate of 63%, and the prevalence in disease-bearing nurseries reached as high as 90%. In comparison to 2013, there had been no improvement in the condition of seedlings affected by CTV. The positive detection rate of citrus yellow vein clearing virus (CYVCV) was found to be 38%, with 70% of the surveyed nurseries exhibiting the disease. The citrus varieties identified as carriers of the disease included 'Qicheng', 'Shatangju', 'Wogan', and 'Gonggan'. Nematodes were isolated from the matrix and roots of seedlings grown in both container and open field environments. The susceptibility of container seedlings to nematodes was found to be 36.4%, while the susceptibility of open field seedlings was 38.6%. Statistical analysis indicated no significant difference in susceptibility between the two groups. [**Conclusions**] The disease detection rates associated with various seedling rearing methods and citrus varieties exhibited notable variability. Open field seedlings without the protection of mesh chambers demonstrated a higher susceptibility to disease. Additionally, the types of infectious diseases varied among the different citrus varieties.

Key words Citrus diseases and pests; Investigation; qPCR; RT-PCR; Detection

1 Introduction

Citrus huanglongbing (HLB) and citrus bacterial canker disease (CBCD) represent the primary agricultural plant quarantine diseases in China. Additionally, citrus tristeza virus (CTV), citrus yellow vein clearing virus (CYVCV), and nematodes are significant diseases and pests affecting citrus crops, which pose a substantial threat to the advancement of the citrus industry. Gao Fanglan *et al.* [1] observed significant variability in the detection rate of HLB across different years, geographical sources, citrus species, and types of symptoms. Based on the resistance and tolerance tests conducted on various citrus varieties in relation to HLB, it was observed that citrus huyou, kumquat, and bergamot exhibited greater resistance to the disease, whereas ponkan and ougan demonstrated higher susceptibility[2]. Zhang Yang *et al.* [3] demonstrated that there were minor variations in the infection rates of CBCD across different citrus species. Specifically, the incidence of infection in navel oranges and pomelos was found to be 4.47% higher than that observed in bingtang oranges. The analysis of 197 samples collected from citrus shatangju orchards in Zhaoqing City and Yunfu City revealed a positive detection rate of 99% for CTV. Additionally, the positive detection rate in citrus nurseries was found to be 77%. These findings indicate that the citrus shatangju in the production areas are predominantly infected with CTV[4]. Liu Shunmin *et al.* [5] conducted an analysis of 272 citrus samples from Taizhou to assess the presence of CYVCV, resulting in a positive detection rate of 34.9%. The investigation into the prevalence of nematode diseases in the primary citrus shatangju-producing regions of Guangdong Province revealed that the incidence of nematode diseases in orchards was 94%, while the incidence among individual plants was 65%. Additionally, some plants exhibited complex infections[6]. To enhance the implementation of source control, gain insights into the development of the citrus industry, and manage the prevalence of diseases and pests, we conducted an investigation into citrus diseases and pests across several locations. This study included 15 citrus nurseries located in eight regions across Zhaoqing, Yunfu, Yangchun, and Meizhou cities in Guangdong Province, as well as Guilin and Nanning cities in Guangxi Zhuang Autonomous Region, and Chongqing. During this investigation, we collected citrus leaf samples, root samples, and matrix samples, which were subsequently transported to the laboratory for analysis. The collected samples were subjected to

Received: July 12, 2024 Accepted: September 25, 2024
Supported by Earmarked Fund for China Agriculture Research System (CARS-26); Science and Technology Innovation Guidance Project of Zhaoqing City (2023040308008); Undergraduate Innovation and Entrepreneurship Training Program of Guangdong Province (S202310580050); Project of High-quality Development in Hundred Counties, Thousands Towns and Ten Thousand Villages.
* Corresponding author. E-mail: qhgee@163.com

various detection methods, including quantitative polymerase chain reaction (qPCR), reverse transcription polymerase chain reaction (RT-PCR), and the Berman funnel method, to assess the presence of diseases and pests. This approach aimed to elucidate the prevalence patterns and control status associated with major diseases and pests within the nurseries.

2 Materials and methods

2.1 Materials The experimental materials were sourced from 15 citrus nurseries situated across 8 regions, specifically within the cities of Zhaoqing, Yunfu, Yangchun, and Meizhou in Guangdong Province, as well as in the cities of Guilin and Nanning in Guangxi Zhuang Autonomous Region, and in Chongqing (Table 1).

Table 1 Sample collection information of citrus nurseries

Nursery code	Sampling location	Variety
1	Deqing County, Zhaoqing City, Guangdong Province	*Citrus nobilis* Lour. 'Gonggan'
2	Deqing County, Zhaoqing City, Guangdong Province	*Poncirus trifoliata* (L) Raf. 'zhike', *C. nobilis* Lour. 'Gonggan', *Fortunella* Swing. 'Cuimijinju', *C. nobilis* Lour. 'Wogan'
3	Deqing County, Zhaoqing City, Guangdong Province	*C. nobilis* Lour. 'Wogan', *C. nobilis* Lour. 'Gonggan'
4	Sihui City, Zhaoqing City, Guangdong Province	*C. nobilis* Lour. 'Wogan', *C. reticulata* cv. 'Jinqiushatangju'
5	Sihui City, Zhaoqing City, Guangdong Province	*C. nobilis* Lour. 'Wogan', *C. reticulata* cv. 'Jinqiushatangju', *C. haniana* Hort. ex Tseng. 'Xinhuigan', *C. flamea* Hort. ex Tseng. 'Shatangju', *C. flamea* Hort. ex Tseng. 'Jinkuimiju'
6	Sihui City, Zhaoqing City, Guangdong Province	*C. flamea* Hort. ex Tseng. 'Shatangju'
7	Sihui City, Zhaoqing City, Guangdong Province	*C. flamea* Hort. ex Tseng. 'Shatangju', *Fortunella* Swing. 'Jinju', *C. nobilis* Lour. 'Gonggan'
8	Xinxing County, Yunfu City, Guangdong Province	*C. nobilis* Lour. 'Gonggan', *C. flamea* Hort. ex Tseng. 'Shatangju', *C. nobilis* Lour. 'Wogan', *C. haniana* Hort. ex Tsen 'Chazhigan', *C. sinensis* Osbeck. 'Hongjiangcheng'
9	Xinxing County, Yunfu City, Guangdong Province	*C. flamea* Hort. ex Tseng. 'Shatangju'
10	Yangchun City, Guangdong Province	*C. flamea* Hort. ex Tseng. 'Shatangju'
11	Meizhou City, Guangdong Province	*C. nobilis* Lour. 'Wogan', *C. sinensis* Osbeck. 'Qicheng', *C. grandis* (L) Osbeck. 'Shatianyou'
12	Guilin City, Guangxi Province	*C. sinensis* Osbeck. 'Qicheng', *C. grandis* (L) Osbeck. 'Shatianyou'
13	Quanzhou County, Guilin City, Guangxi Province	*C. nobilis* Lour. 'Wogan', *C. sinensis* Osbeck. 'Qicheng'
14	Nanning City, Guangxi Province	*C. nobilis* Lour. 'Wogan'
15	Chongqing City	*C. nobilis* Lour. 'Wogan', *C. flamea* Hort. ex Tseng. 'Jinkuimiju', *C. sinensis* Osbeck. 'Qicheng', *C. nobilis* Lour. 'Youliangmiju'

2.2 Methods

2.2.1 Sampling. Samples of HLB, CBCD, CTV, and CYVCV were randomly selected for analysis. For each sample, five mature leaves from the middle and upper portions of the seedlings were selected. The samples were then rapidly frozen in liquid nitrogen and transported back to the laboratory in a dry ice box.

Nematodes were randomly sampled from container seedlings. A total of 6 g of lateral roots and 150 g of rhizosphere soil from seedlings were collected and transported to the laboratory in small self-sealing bags for the purpose of nematode isolation. Nematodes were randomly sampled from open field seedlings. The roots of each seedling were excavated at a diagonal angle, and the lateral roots (6 g) and rhizosphere soil (150 g) of citrus trees were transported back to the laboratory for the isolation and detection of nematodes.

2.2.2 Detection methods. The detection of HLB was conducted following the methodology established by Ren Suli[7]. The detection of CBCD adhered to the procedures outlined by Zhao Yun[8]. The detection of CTV was performed in accordance with the method described by Ding Fang[9]. Additionally, the detection of CYVCV was executed based on the approach proposed by Chen Hongming[10]. Nematode detection was carried out utilizing the Berman funnel method[11].

3 Results and analysis

3.1 HLB A total of 145 samples, comprising diverse varieties, were analyzed, and 5 positive samples were identified, representing 3% of the total. One out of 15 citrus nurseries was found to be infected with HLB, representing 6% of the total number of nurseries sampled. A total of 91 container seedling samples and 54 open field seedling samples were collected for analysis. The detection rate of HLB in the container seedling samples was found to be 0%, while the detection rate in the open field seedling samples was 9% (Fig. 1). Notably, all positive samples originated from a nursery located in Deqing County, Zhaoqing City.

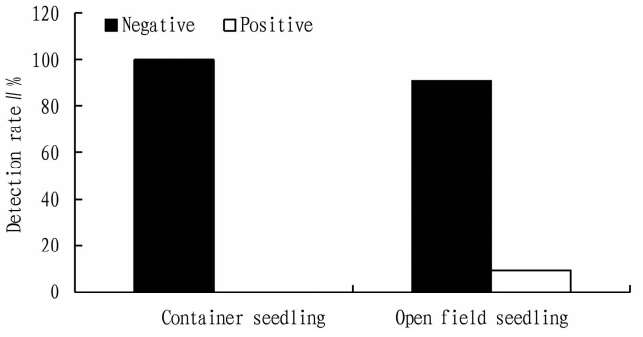

Fig. 1 Detection results of HLB

In 2013, we conducted an investigation into the state of nurseries within the citrus industry zone of the Xijiang River Basin in Guangdong Province. The findings revealed that there was only one container seedling nursery equipped with mesh chambers, located in Sihui, while the remaining nurseries operated as open field seedling establishments without mesh chambers. These nurseries predominantly employed traditional techniques and lacked mother trees and cutting orchards. Most scions were sourced from pruned branches of well-growing orchards, which poses a significant risk to the production of seedlings in the region. The significant outbreak of HLB has had a profound impact on the citrus industry. Many small nursery farms, which played a crucial role in the large-scale expansion of citrus cultivation in the region, have ceased operations. Additionally, the remaining nursery farms have experienced a substantial reduction in size compared to their previous capacities. The seedlings were surveyed and sampled from seedling enterprises within mesh chambers, the majority of which were container seedlings, while a limited number were cultivated in open field conditions. The scope of the survey was broadened to include virus-free nurseries located in various regions of the province as well as in certain neighboring provinces, in addition to the citrus industrial zone situated in the Xijiang River Basin of Guangdong Province. Currently, there are three virus-free nurseries established within the citrus industrial zone of the Xijiang River Basin in Guangdong Province. Among these, two nurseries are high-standard facilities dedicated to the breeding of virus-free container seedlings. Notably, none of these nurseries have reported the presence of HLB. The establishment of standardized nurseries has progressed to a certain degree, and there has been a notable focus on the breeding of virus-free seedlings. However, a case of contamination was identified in a nursery that was under construction by other enterprises, indicating the presence of ongoing latent risks. Several nurseries sampled from various regions within the province and from neighboring provinces did not detect the presence of HLB.

3.2 CBCD Among the 145 samples analyzed, 24 positive samples were identified, representing 16.5% of the total. The analysis included 15 nurseries, of which 5 tested positive, constituting 33% of the total nurseries examined. The positive samples originated from one nursery in Deqing, three nurseries in Sihui, and one nursery in Guangxi. Additionally, three virus-free nurseries located in the Xijiang River Basin of Guangdong Province did not exhibit any detectable presence of CBCD (Table 2).

Table 2 Detection results of CBCD in 15 citrus nurseries

Nursery code	Detection result	Nursery code	Detection result
1	Negative	9	Negative
2	Positive	10	Negative
3	Negative	11	Negative
4	Positive	12	Negative
5	Positive	13	Positive
6	Positive	14	Negative
7	Negative	15	Negative
8	Negative		

In relation to the various citrus varieties presented in Table 3, a total of 13 distinct citrus varieties were collected. Among these, 'Wogan', 'Shatangju', 'Jinqiushatangju' and 'Jinkuimiju' were identified as being infected with CBCD, with the prevalence of these varieties amounting to 30.7%. No positive samples were identified in the three established virus-free nurseries located in the Xijiang River Basin of Guangdong Province. However, other nurseries currently under construction by various enterprises exhibited a high detection rate, comprising 80% of the total samples tested. This included a detection rate of 60% in Sihui nurseries and 20% in Deqing nurseries. An analysis of the underlying factors reveals that these nurseries predominantly utilize open field nurseries without mesh chambers. Furthermore, the source of scions is primarily self-propagated. The spread of the CBCD occurs mainly to neighboring plants via various vectors, including wind, rain, insects, as well as through branches and leaves. The disease is transmitted in the form of germ-carrying seedlings and scions. Hence, the primary methods for disease prevention include the standardization of seedlings and scion seedlings, the construction of mesh chambers, and the planting of virus-free container seedlings.

Table 3 Detection results of CBCD in different citrus varieties

No.	Variety	Detection result			
		Negative %	Nursery code	Positive %	Nursery code
1	'Gonggan'	100	1,2,3,7,8	0	
2	'Zhike'	100	2	0	
3	'Cuimijinju'	100	2	0	
4	'Wogan'	45	3,4,8,11,14	55	2,4,5,13
5	'Jinqiushatangju'	65	4	37	5
6	'Jinkuimiju'	20	15	80	5
7	'Xinhuigan'	100	5	0	
8	'Shatangju'	85	5,7,8,9,10,12	15	6
9	'Jinju'	100	7	0	
10	'Chazhigan'	100	8	0	
11	'Qicheng'	100	12,13,15,11	0	
12	'Youliangmiju'	100	15	0	
13	'Shatianyou'	100	11	0	

3.3 CTV The analysis of 29 citrus samples obtained from 10 nurseries revealed that 18 samples tested positive for CTV, resulting in an overall detection rate of 63%. Furthermore, CTV was detected in 9 out of the 10 nurseries, yielding a detection rate of 90% (Tables 4 – 5). A total of 16 samples were collected from

the Xijiang River Basin in Guangdong Province, of which 12 tested positive for CTV, resulting in a detection rate of 75%. In other regions of Guangdong Province, 4 samples, comprising different varieties, were collected, with 1 sample testing positive, which constituted 25% of the total. Additionally, in several neighboring provinces, 9 samples, comprising diverse varieties, were collected, and 5 of these samples tested positive, accounting for 55% of the total.

Table 4 Detection results of CTV in 10 citrus nurseries

Nursery code	Detection result	Nursery code	Detection result
1	Positive	11	Positive
2	Positive	12	Positive
3	Positive	13	Positive
8	Positive	14	Positive
10	Negative	15	Positive

Table 5 Detection results of CTV in different citrus varieties

No.	Variety	Detection result			
		Negative %	Nursery code	Positive %	Nursery code
1	'Gonggan'			100	1,2,3,8
2	'Zhike'	100	2		
3	'Cuimijinju'	50	2	50	2
4	'Wogan'	44	13,15,2	55	3,8,11,13,14
5	'Hongjiangcheng'			100	8
6	'Jinkuimiju'	100	15		
7	'Shatangju'	100	10,12,8		
8	'Chazhigan'			100	8
9	'Qicheng'			100	11,12,15
10	'Youliangmiju'			100	15
11	'Shatianyou'	100	11		

In the 2013 research report, a total of 22 samples collected from 16 nurseries located in Qingyuan, Sihui, Deqing, and Yunfu were analyzed using direct tissue blot immunoassay (DTBIA). Among these samples, 17 tested positive for CTV, resulting in a detection rate of 77%[4]. In this study, RT-PCR was employed to identify CTV, which encompasses stem pitting tristeza. The findings from the two tests indicated that there was no improvement in the condition of seedlings affected by CTV within the nurseries of this production area. Furthermore, nurseries located in neighboring regions and outside the province also exhibited a high prevalence of the disease. The occurrence of stem pitting tristeza in citrus cultivation poses significant risks to the healthy development of the industry. Consequently, seedlings that harbor the disease have become an important consideration in the overall production process.

3.4 CYVCV A total of 29 samples were collected from 10 nurseries, and the results indicated that 7 nurseries tested positive, representing 70% of the samples. The positive samples included 'Qicheng', 'Shatangju', 'Wogan' and 'Gonggan'. Out of 29 samples analyzed, 11 were found to be positive, representing 38% of the total (Tables 6 – 7). Specifically, 16 samples were collected from the citrus industrial zone located in the Xijiang River Basin of Guangdong, of which 7 tested positive, constituting 44% of that subset. The positive samples included 'Shatangju', 'Wogan' and 'Gonggan'. A total of 4 samples were collected from other areas in Guangdong Province, of which one was positive, representing a 25% positivity rate. Additionally, 9 samples were collected from neighboring provinces, with 3 yielding positive results, accounting for 33% of that group. During the nursery survey, the leaves appeared healthy to the naked eye, exhibiting no symptoms of CYVCV.

Table 6 Detection results of CYVCV in different citrus varieties

No.	Variety	Detection result			
		Negative %	Nursery code	Positive %	Nursery code
1	'Gonggan'	17	1	83	1,2,3,8
2	'Zhike'	100	2	0	
3	'Cuimijinju'	100	2	0	
4	'Wogan'	11	3	89	2,8,11,14,15
5	'Hongjiangcheng'	100	8		
6	'Jinkuimiju'	100	15		
7	'Shatangju'	67	10,12	33	8
8	'Chazhigan'	100	8	0	
9	'Qicheng'	50	12	50	15
10	'Youliangmiju'	100	15	0	
11	'Shatianyou'	100	11	0	

Table 7 Detection results of CYVCV in 10 citrus nurseries

Nursery code	Detection result	Nursery code	Detection result
1	Positive	11	Negative
2	Positive	12	Positive
3	Positive	13	Negative
8	Positive	14	Positive
10	Positive	15	Negative

3.5 Nematode In the current study (Fig. 2), nematodes were isolated from the substrate and root systems of both container-grown and open field seedlings. The susceptibility of container seedlings was found to be 36.4%, while that of open field seedlings was 38.6%, indicating minimal variation between the two groups. There exists a diverse array of nematode species, among which the citrus root nematode is particularly detrimental to citrus cultivation. This nematode is prevalent in the primary citrus-producing regions across various provinces in China. Notably, *Tylenchulus semipenetrans* and *Meloidogyne* spp. are identified as the most harmful species. In this survey, the total number of nematodes was isolated, and the morphological classification of certain nematode samples indicated the presence of *T. semipenetrans* and *Meloidogyne* spp. The findings of the study indicated that nematodes were more prevalent in nursery substrates or soil. Firstly, certain nurseries procure their own raw materials for preparation without imple-

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

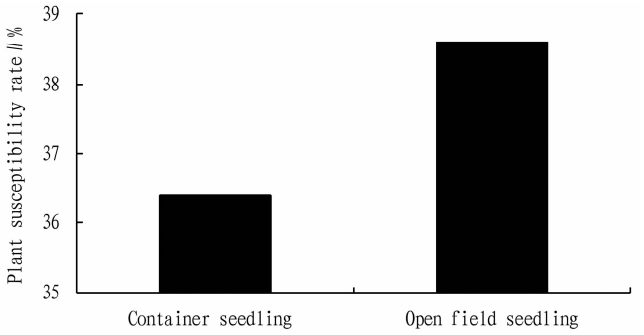

Fig. 2 Results of nematode plant susceptibility in citrus nurseries

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.

References

[1] GAO FL, GENG XX, ZHANG MQ. Present status of citrus Huanglongbin and genetic diversity of *Candidatus* Liberibacter asiaticus in Guangxi[J]. Journal of Southern Agriculture, 2023, 54(1): 128 – 138. (in Chinese).

[2] JIANG ZZ, YUAN YW, MENG YQ, *et al.* Resistance test of different citrus varieties to citrus Huanglong disease[J]. China Plant Protection, 2006(9): 23 – 24. (in Chinese).

[3] ZHANG Y, DAI SM, LONG GY, *et al.* Investigation on the occurrence and control status of main citrus diseases and pests in Hunan[J]. Hunan Agricultural Sciences, 2021(12): 61 – 64. (in Chinese).

[4] GUO LY, GUO YJ, JI QH, *et al.* Detection of citrus recession virus in the main production area of satsuma orange in Guangdong Province, China [J]. South China Fruits, 2015, 44(2): 43 – 44. (in Chinese).

[5] LIU SM, LU J, PU ZS, *et al.* Occurrence and distribution of citrus yellow vein virus in Taizhou and its molecular characteristics[J]. Zhejiang Ganju, 2024, 41(1): 12 – 20. (in Chinese).

[6] JIANG H, GUO YJ, JI QH, *et al.* Preliminary investigation on the occurrence of nematode disease in the main production areas of satsuma oranges in Guangdong Province[J]. South China Fruits, 2015, 44(2): 54 – 56, 59. (in Chinese).

[7] REN SL. Studies on the interactions between citrus-Huanglong disease pathogens-Wolbachia-citrus woodlouse[D]. Guangzhou: South China Agricultural University, 2016. (in Chinese).

[8] ZHAO Y. Research on quantitative PCR inspection and quarantine technology of citrus ulcer disease[D]. Chongqing: Chongqing University, 2006. (in Chinese).

[9] DING F, CAO Q, WANG GP, *et al.* Studies on the simultaneous detection of citrus Huanglongbing pathogen, citrus exocortis viroid, citrus tristeza virus by multiplex RT-PCR[J]. Acta Horticulturae Sinica, 2006, 33(5): 947 – 952. (in Chinese).

[10] CHEN HM, WANG XF, ZHOU Y, *et al.* Biological characterization and RT-PCR detection of a new disease of Eureka lemon[J]. Journal of Plant Protection, 2015, 42(4): 557 – 563. (in Chinese).

[11] General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China. SN/T 2757-2011. Rules for detection of plant parasitic nematodes[S]. Beijing: Standards Press of China, 2011. (in Chinese).

(From page 14)

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^2, thereby enhancing the overall treatment effectiveness.

References

[1] ZHONG WM, CUI SP. The spread and impact of tobacco in China in the context of modern globalization[J]. Seeking Truth, 2020, 3: 157 – 170. (in Chinese).

[2] CUI XH. Development status, characteristics and future prospects of China's tobacco industry[J]. Business Research, 2021, 29: 8 – 10. (in Chinese).

[3] ZHU XC, WANG YT, WANG ZF, *et al.* Tobacco Diseases of China [M]. Beijing: China Agricultural Publishing House, 2002: 21 – 36. (in Chinese).

[4] LI CB, CHENG YL, HE Y, *et al.* High efficiency fungicide screening for *Phytophthora parasitica* var. *nicotianae*[J]. Tobacco Science & Technology, 2023, 56(6): 34 – 39. (in Chinese).

[5] HE L, LI JM, LEI MX, *et al.* Combined toxicity of Metalaxy-M mixed fludioxonil to *Phytophtora parasitica* var. *nicotianae*[J]. World Pesticide, 2016, 38(2): 52 – 53, 61. (in Chinese).

[6] CHANG JB, CHEN YG, MIAO SY, *et al.* Field control effect of pesticides against tobacco black shank[J]. Journal of Zhejiang Agricultural Sciences, 2022, 63(11): 2649 – 2651. (in Chinese).