Occurrence Dynamics of Citrus Target Spot in Wanzhou District of Chongqing City and Compound Fungicide Screening of *Pseudofabraea Citricarpa*

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Abstract: To explore the occurrence dynamics and infection characteristics of citrus target spot, and screen effective fungicides and provide data support for citrus safe production. Two orchards with different altitudes and different management levels in Shuangshi community and Baiyan Village of Wanzhou District Chongqing city were selected to follow up the occurrence of citrus target spot. At the same time, the inhibition effects of 6 compound fungicides to *Pseud of abraea Citricarpa* (Citrus Target spot) were determined by the mycelial growth rate inhibition method. The results showed that, in December of 2021 to March of 2022, in the 2 orchards, the average disease spot, number of falling leaf showed a trend of reduce after the first rise, and incidence and disease index were characterized by rising gradually along with the passage of time. From late to the end of January, there were more rainy and foggy days, and the temperature dropped sharply, which aggravated the defoliation. Orchard 1 was with higher altitude 750 m and loose management, and the disease outbreak came more rapidly and severely. While in orchard 2, the lower altitude, higher management level and stronger tree made the disease be later and lighter than orchard 1. Further studies of compound fungicide screening showed that the EC_{50} against *Ps. Citricarpa*, from low to high, was 40% Tebuconazole·Prochloraz (0.03295)<13% Thifluzamide·Hexaconazole (0.03634)<30% Difenoconazole·Propiconazole (0.03881)<40% Difenoconazole·Pyraclostrobin (0.30849)<30% Thifluzamide·Tebuconazole (0.90167)<33 % Kasugamycin·Copper Quinoline (2.92935). These fungicides can be used for further research in the field test.

1 INTRODUCTION

China is the largest citrus producer in the world, and citrus in our country are planted about 2.67 million hm^2 in area, the output is 51 million tons (Wu, 2018; Li, 2011). Target spot was a new leaf-spotting fungal disease of citrus caused by *Pseudofabraea citricarpar* (Chen, 2016; Ao, 2021; Liu, 2015; Luo, 2015), which has caused considerable economic losses in local citrus production (Yang, 2018). This fungal pathogen was first reported infecting both Satsuma mandarin (*Citrus unshiu* Marcov.) and kumquat (*Fortunella margarita (*Lour.) Swingle) in Shanxi province, China, in 2004 (Zhu, 2011; Zhu, Wang, 2012; Zhu, 2012). Subsequently, the disease was found to infect more citrus varieties, such as 'Ponkan'mandarin (*C. reticulata* Blanco var. Ponkan) in Hunan Shanxi province, China, 'Eureka' [*C. limon* (L.) Burm.F.] 'Beijing'lemon (*C. meyerii* Y. Tan.), 'Newhall'navel (*C.sinensis*) orange and Tarocco blood orange (*C. sinensis* (L.) Osbeck var. Tarrocco) in Chongqing city, et al (Xiao, 2020; Zhan, 2021). Citrus target spot pathogen *Pseudofabraea citricarpa* will not only infect citrus leaves and branches, but also cause fruits with about 0.5cm-2.2cm brown or black spots. Severely affected leaves and fruits drop easily, resulting in tree defoliation.

In recent years, citrus target spot occured more frequently in our country, showing a trend accelerating from the north to the south. This disease has the greatest potential harm to the two major citrus-producing areas in the Yangtze River Basin and in western Hubei and western Hunan (Xu, 2020). In the Three Gorges reservoir area of Chongqing, the late-ripening citrus has a long growth cycle, which the fruits were hung in the trees over winter and ripens in late March of the next year. This led to higher risk of the fruits.

So far, studies on citrus target spot mainly focused on etiology (Zhu, Wang, 2012; Zhu, 2012; Xiao, 2020; Zhan, 2021), molecular detection (Yang, 2018), prediction of suitable areas (Xu, 2020), resistance identification (Chen, 2022), etc. There were no reports on the disease occurrence dynamics, and there were no effective compound agents, which seriously affects the taken of disease control by plant protection workers. Therefore, it is necessary to research the occurrence dynamics, screen effective agents, so as to take effective

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control measures against the disease and to prevent the further spread of citrus target spot.

2 METHODOLOGY

2.1 Places and Objects of Investigation

The experiment was conducted in two orchards with different altitudes and management levels in Baiyan Village and Shuangshi Community of Baiyang Town, Wanzhou District, Chongqing City. Baiyan Village (Orchard 1): It with 34.33 hm^2 at an elevation of 410 m, which was planted 22,000 'Eureka' lemon [*C. limon* (L.) Burm.F.]. They were all 10 years old. Shuangshi Community (Orchard 2): Covering an area of 0.2 hm^2 at an elevation of 750 m, and Similarly, there were 132 'Eureka' lemon trees [*C. limon* (L.) Burm.F.] which were 10 years old. At the end of November, the two orchards were treated by lime sulphur with 0.5 baume degree.

2.2 Investigation Method

In each orchard, the 6 'Eureka' lemon trees were selected diagonally and randomly in the shape of "Z". Each tree was divided into all four directions. Each branch and its' all leaves were listed and numbered. The number of diseased spots per leaf, fallen leaves, disease-free leaves, the incidence and the disease index were counted. The survey dates were respectively December 1 st, December 12 th, December 29 th, January 5 th, January 12 th, January 19 th, January 26 th, February 2 nd, February 9 th, February 16 th, February 23 th, March 2 th, March 9 th and March 16 th in 2021, with a total of 14 times of survey. Incidence was obtained using the formula: Incidence (%) = (infected leaves/inoculated leaves) ×100 % (Chen, 2022). Disease index was obtained using the formula: Disease index = [Σ (number of every grade × grade) / inoculated leaves × 9) × 100 (Wang, Cao, 2020).

Standards for disease severity grading was as below. Grade 0: no spot. Grade 1: the lesion area accounted for less than 5% of the whole leaf area. Grade 3: the lesion area accounted for 5 % ~ 10 % of the whole leaf area. Grade 5: the lesion area accounted for 11 % ~ 25 % of the whole leaf area. Grade 7: the lesion area accounted for 26 % ~ 50 % of the whole leaf area. Level 9: The lesion area accounts for more than 50 % of the whole leaf area or the leaf falls.

2.3 Fungal Pathogens

Ps. citricarpa strains WZSS 5 was isolated from 'Eureka' lemon leaves in Wanzhou District, Chongqing City. The strain were incubated on PDA medium at 20 °C until the mycelium covered approximately three-quarters of the plates (about 15 to 20 days) and stored at 4 °C for further study.

2.4 Fungicides

The fungicides tested include in table 1.

Table 1. Details of the 6 fungicides tested.

Reagent name	Effective content and dosage form	Manufacturer
Thifluzamide · Tebuconazole	30 % suspending concentrate	Jiangxi Tianyou Biochemical Co., LTD
Tebuconazole · Prochloraz	40 % microemulsion	Guangxi Huifeng Biotechnology Co., LTD
Difenoconazole · Propiconazol	60 % emulsifiable concentrates	Anhui Meilan Agricultural Development Co., LTD
Difenoconazole · Pyraclostrobin	40 % suspending concentrate	Shandong Dongtai Agrochemical Co., LTD
Kasugamycin · Copper Quinoline	33 % suspending concentrate	Shandong Yilan Science and Technology Co., LTD
Thifluzamide · Hexaconazole	13 % suspending concentrate	Anhui Sida Pesticide Chemical Co., LTD

2.5 Fungicide Test Method

The toxicity of fungicides was determined by the mycelial growth rate method (Zhan, 2021). The fungicides were mixed into 1 g/10 ml mother liquor, then diluted into various concentration, added into the melted potato dextrose agar (PDA) in a certain proportion, mixed, and poured into the petri dishes. Briefly, an individual sclertium square (5 × 5 mm) were placed onto the center of the PDA and cultured in a 20 °C. PDA medium was used as a control. After 7 days, the diameter of hypha was measured by the cross-over method, and the inhibition rate of fungicide on hypha growth was calculated (Yang, 2011). All treatments were repeated 3 times. Hyphal growth inhibition rate (%) = [(colony diameter of control- colony diameter of treated)/ colony diameter of control] × 100 %. The Toxic regression equation, correlation coefficient (R^2) and median effective concentration (EC_{50}) were calculated (Yang, 2011).

2.6 Statistical Analysis

The data was analyzed by IBM SPSS 16.0 (New York) and Microsoft Excel.

3 TEST RESULTS

3.1 Occurrence Dynamics of Citrus Target Spot

The number of leaves and fallen leaves in Orchard 1 were stable before January 12th, and there was no There was no significant defoliation. Subsequently, from January 12 th to February 9 th, and severe defoliationa was occurred. And then, the falling peak was reached on January 26 th (135 fallen leaves) (Figure 1). The number of diseased spots also increased sharply along with the survey. On January 19 th, the total number of diseased spots and the average number of diseased spots per leaf were the maximum, which were 3448 and 11.97 respectively (Figure 2). The incidence rate increased rapidly from 15.77 % conducted on December 1 st to 100 % on January 19 th (Figure 3). The disease index showed a gradual increase over time, reaching a maximum of 59.59 (Figure 4). From now on, the total number of diseased spots, the average number of diseased spots per leaf and the number of fallen leaves gradually decreased, and there were only 22 leaves in the fixed branches of investigation until March 16 th. At this moment, the falling rate was of 92.62 % and the incidence was 100 % (Figure 1 and Figure 3).

Orchard 2 showed a similar pattern to Orchard 1, but the disease progres developed more slowly. Until the end of the investigation, the defoliation rate was also only 25.71 % (Figure 1). The total number of disease spots was less than Orchard 1 as well and reached the maximum of 933 on February 23 th. Before January 12, the average number of diseased spots per leaf was less than 1. During the investigation period, the number of diseased spots per leaf was mostly less than 5, and that was above 16 was almost 0 (Figure 2). The incidence rate was below 50 % before January 19 th, and following, it was gradually increased, of which was reaching a peak of 93.59 % on March 16 th (Figure 3). The disease index peaked at 24.77 on February 9th (Figure 4).

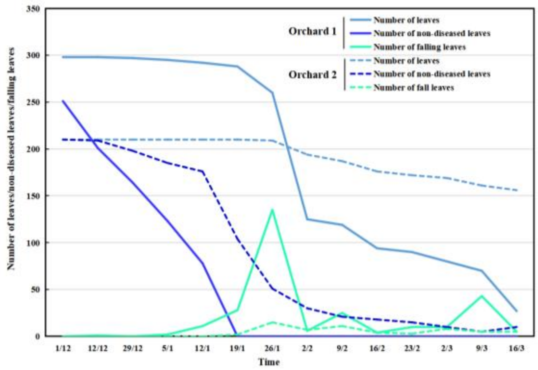

Figure 1 Occurrence dynamics of citrus target spot in the two orchards

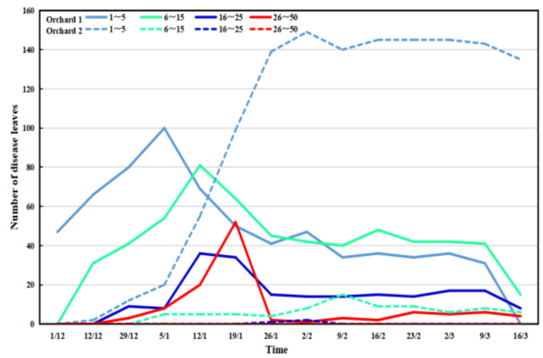

Figure 2 Change of the number of lesions of leaf in different time in the two orchards

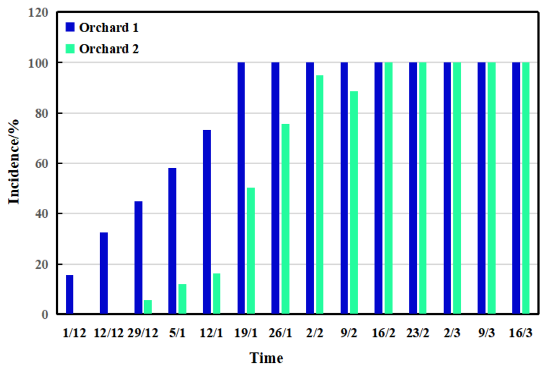

Figure 3 Change of the incidence on leaf in different time in the two orchards

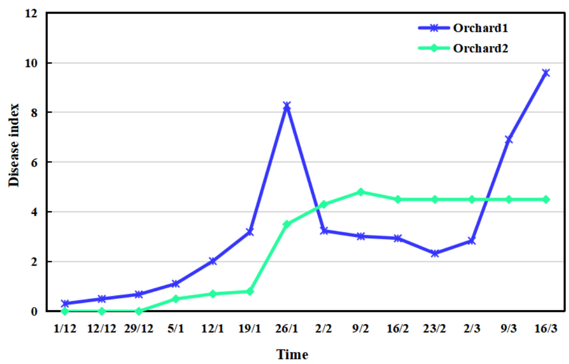

Figure 4 Change of the disease index of citrus leaf in different time in the two orchards

3.2 Meteorological Factors During the Survey

According to Figure 5 and Figure 6, during the investigation period, the rain days accumulated for 48 d (accounted foe 39.67 %), fog days accumulated for 3 d, and the total rain and fog days accounted for 66.94 %. At the same time, the days of low temperature below 10 ℃ was got to 100 d, accounting for 82.65 % of days during investigation Furthermore, the days of low temperature below 5 ℃ was for 46 d, accounting for 38.01 %. Of which, according to the data of the meteorological department, it was raining for 16 days in January. Moreover, from January 20 th to 29 th, it was raining for 9 days, accompanied by the lowest temperature of 1 ℃ - 7 ℃, which aggravated the falling of diseased leaves during this period.

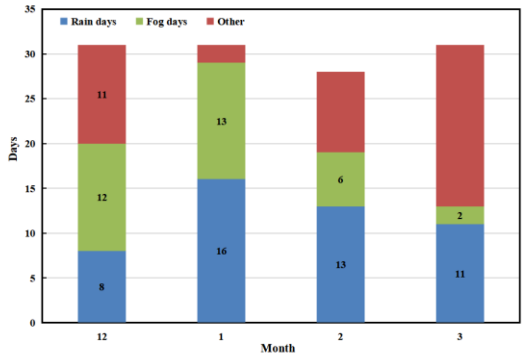

Figure 5 Rain and fog days during surveys

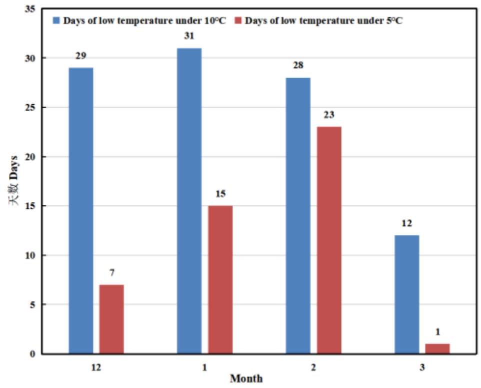

Figure 6 Days of low temperature under 10 ℃ and 5 ℃ during the surveys

3.3 Toxicity of Fungicides Against *Ps. citricarpa*

The results showed that 6 kinds of compound fungicides all had inhibitory effects on the growth of *Ps. citricarpa* in different degrees, but there were differences among various fungicides. In the all test fungicides, the EC_{50} against *Ps. Citricarpa*, from low to high, was 40% Tebuconazole · Prochloraz < 13% Thifluzamide · Hexaconazole < 30% Difenoconazole · Propiconazole < 40% Difenoconazole· Pyraclostrobin < 30% Thifluzamide·Tebuconazole < 33% Kasugamycin·Copper Quinoline. In addition, 40% Tebuconazole · Prochloraz has the best inhibition effect among them, of which the EC_{50} was 0.03295 μg/mL. At the same time, EC_{50} of 40% Difenoconazole· Pyraclostrobin were respectively 9.36, 8.49 and 7.95 times of 40% Tebuconazole · Prochloraz, 13% Thifluzamide · Hexaconazole and 30% Difenoconazole.Propiconazole. EC_{50} of 30% Thifluzamide·Tebuconazole were respectively 27.36, 24.81 and 23.23 times of 40% Tebuconazole · Prochloraz, 13% Thifluzamide · Hexaconazole and 30% Difenoconazole.Propiconazole. Whereas, EC_{50} of 33% Kasugamycin·Copper Quinoline were got respectively 88.90, 80.61 and 75.47 times of the above three test agents (Table 2).

Table 2. Comparisons of virulence of 6 fungicides to *Pseudofabraea Citricarpa*

Test agents	Toxicity regression equation	Correlation coefficient (R^2)	EC_{50} (μg/mL)
40 % Tebuconazole · Prochloraz	Y = 0.3927 x + 5.582	0.9444	0.03295
13 % Thifluzamide · Hexaconazole	Y = 0.2813 x + 5.418	0.9527	0.03634
30 % Difenoconazole.Propiconazole	Y = 0.2466 x + 5.347	0.8290	0.03881
40 % Difenoconazole· Pyraclostrobin	Y = 0.4184 x + 5.213	0.9533	0.30849
30 % Thifluzamide·Tebuconazole	Y = 0.3782 x + 5.017	0.9028	0.90167
33 % Kasugamycin·Copper Quinoline	Y = 0.6982 x +4.6741	0.9582	2.92935

4 DISCUSSION

Through the investigation of two orchards with different altitudes and management levels in Baiyang Town, Wanzhou District, Chongqing from December, 2021 to March 2022, the results showed that different altitudes affected the microclimate of orchards, and different management levels affected trees' strength and resistance. The incidence of the disease in the two orchards broke out with the decrease of temperature and the increase of rain and fog. By the end of March, almost all the leaves were fallen. However, there were significant differences in the incidence rate and severity between the two orchards, indicating that temperature and rain may determine the occurrence and severity of citrus target spot. Therefore, the orchards in high-altitude areas should be treated with protective agents in early November and early January in low-altitude areas to prevent disease. Potassium fertilizer should be added in autumn to improve disease resistance (Peng, 2016) and improve cold resistance of citrus. Late-ripening citrus fruits, in particularly, should be carefully protected.

Chemical fungicides are used to control citrus ring spot in the field (Liu, 2015; Zhu, Wang, 2012; Zhu, 2012). Zhu Li et al. found that the Difenoconazole and prochloraz on citrus target spot were effective in the laboratory Inhibition effect (Zhu, Wang, 2012; Zhu, 2012). This experiment was conducted by laboratory toxicity test, the results indicated that 40 % Tebuconazole·Prochloraz, 13%

Thifluzamide · Hexaconazole, 30% Difenoconazole. Propiconazole had better inhibition effect for the growth of the strain, which can be used for further research in the field test.

5 CONCLUSION

The altitude, climate factors and management level of orchards all had effects on the infection of target spot. The orchards in high altitude area appeared symptoms of the disease earlier and had more leaf spots. In case of continuous rain and temperature reduction, more severe defoliation was accompanied. 40% Tebuconazole · Prochloraz, 13% Thifluzamide · Hexaconazole, 30% Difenoconazole · Propiconazole had better inhibition effect for the growth of *Ps. citricarpa*.

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REFERENCES

1. Ao Y., Ding D. K., Deng J. R., Yu X. L., Luo L., "The occurring regularity and control strategies of citrus target spot," China Fruit News 38 (7), 64 - 66 (2021) .
2. Chen C., Gerard J., Verkley M., Sun G., Groenewald J. Z., Crous P. W., "Redefining common endophytes and plant pathogens in Neofabraea, Pezicula, and related genera, " Fungal Biology 120(11), 1291-1322 (2016).
3. Chen Quan, Xu Yonghong, He Jinhui, Yang Yuheng. "Establishment of an identification method of citrus resistance to target spot, "Journal of Fruit Science 39(2), 295 - 301 (2022).
4. LI W. Q., "Composition of worldwide citrus trade in recent years and outlook of Chinese citrus exportation," Agricultural Outlook 7 (6), 51-54 (2011).
5. Liu Y. H., Tian W., Wei G. Y., "Characteristis and control measures of citrus target spot," Guonongzhiyou (5), 36 (2015).
6. Luo L., Zhang B., Zhang Y. H., Huang Y. L., "A new disease of citrus in south of Shanxi-Citrus target spot," China Fruit News 32 (7), 55 - 56 (2015).
7. Peng H. X., Wei X. Y., Xiao Y. X., Sun Y., Biggs A. R., Gleason M., Sheng P. S., Zhu M. Q., Guo Y. Z., Sun G. Y., "Management of Valsa canker on apple with adjustments to potassium nutrition, " Plant Disease 100(5), 884-889(2016).
8. Wang Xuanxuan, Cao Lixin, Xiang Shunde, Chen Jianghua, Cheng Jiasen, Lin Yang, Xie Jiatao, Fu Yanping. " Occurrence dynamics of citrus melanose during winter in Hubei and biological characteristics of pathogen, " Journal of Huazhong Agricultural University 39 (3), 38 - 44 (2020).
9. Wu G. H. A, Terol J., Ibanez V., López-García A., Pérez-Román E., Borredá C., Domingo C., Tadeo F. R., Carbonell-Caballero J., Alonso R., Curk F., Du D. L. ,Ollitrault P., Roose M. L., Dopazo J., Gmitter F. G., Rokhsar D. S., Talon M. "Genomics of the origin and evolution of Citrus," Nature, 554, 7692 (2018).
10. Xiao X. E., Zeng Y. T., Wang W., Cheng L., Qiao X. H., Hou X., LI H. Y., "First report and new hosts of Pseudofabraea citricarpa causing citrus target spot in China," Plant Health Progress 22 (1), 26 - 30 (2020).
11. Xu Y. H., Chen L., Tang S., Ding D. K., Yang Y. H., "Prediction of suitable area and risk analysis for citrus target spot," Scientia Agricultura Sinica 53 (21), 4430 - 4439 (2020).
12. Yang Y. H., Hu J. H., Chen F. J., Ding D. K., Zhou C. Y., "Development of a SCAR Marker-Based Diagnostic Method for the Detection of the Citrus Target Spot Pathogen Pseudofabraea citricarpa," BioMed research international 7128903 (2018).
13. Yang Q. L., Sang L. M., Sun J. R., Ji Z. Q., Yuan W. L., Guo Y. W., Gai Y. X., "Current situation of fertilizer use in China and the method to improve chemical fertilizer utilization efficiency," Journal of Shanxi Agricultural Sciences 39 (07), 690 - 692 (2011).
14. Zhan S., Wu W., Hu J. H., Wu Y. Z., Qiao X. H., Chen L., Cheng L., Zhou Y. "Pathogen identification and screening of control agent of suspected citrus target spot in Wanzhou, Chongqing," Fruit Trees in Southern China 50 (1), 1 - 7(2021).
15. Zhu L., Ding D. K., Li H. Y., "A leaf spot disease of citrus caused by a new species in Cryptosporiopsis," 2022 Annual Meeting of Mycological Society of China, 66 - 67 (2011).
16. Zhu L., Wang X., Huang F., Zhang J., Li H. Y., "A destructive new disease of citrus in China caused by Cryptosporiopsis citricarpa sp. Nov," Plant Disease 96, 804 - 812 (2012a.)
17. Zhu L., "Identification of five pathogens causing citrus disease in China," [Ph. D. Dissertation], Zhejiang University, 1 - 40 (2012).