forests

Article

Development of Integrated Control for Verticillium Wilt of Smoke Trees in Beijing

Bimeng Li [1], Ruifeng Guo [1], Yize Zhao [1], Qiyan Li [1], Lizhou Song [2], Chong Shen [3], Chenming Du [3], Yuntao Gu [3], Guanghang Qiao [4], Liping Wang [3], Fei Yuan [5], Sanxiang Huang [5] and Yonglin Wang [1,*]

1 Beijing Key Laboratory for Forest Pest Control, College of Forestry, Beijing Forestry University, Beijing 100083, China; libimeng1@163.com (B.L.)
2 Beijing Xiangshan Park Administrative Office, Beijing 100093, China
3 Management Department of Badaling Forest Farm, Beijing 102112, China
4 The Institute of Environment and Plant Protection, Beijing Agricultural and Forestry Academy of Sciences, Beijing 100097, China
5 Municipal Forestry and Parks Resource Conservation Center, Beijing 100013, China
* Correspondence: ylwang@bjfu.edu.cn

Abstract: Smoke tree (*Cotinus coggygria*) is an important ornamental tree that represents the autumnal landscape of red leaves in Northern China, especially in Beijing. However, Verticillium wilt, caused by the fungus (*Verticillium dahliae*), has resulted in a high mortality rate for smoke trees, posing a serious threat to the highly valued landscape of red leaves in Beijing. To explore an efficient control measure for Verticillium wilt, we systematically analyzed the applicability and efficacy of multiple treatments for three consecutive years in Xiangshan Park and Badaling Forest Park. From 2021 to 2023, diseased smoke trees in Xiangshan Park were subjected to three application methods (agent irrigation, trunk injection, or a combination of the two) and five candidate agents, namely *Bacillus subtilis*, azoxystrobin, propiconazole, carbendazim, and prochloraz. Analyses of the data for three consecutive years revealed a decreasing trend in the annual disease incidence rate. Specifically, the combined application of agent irrigation and trunk injection exhibited the highest control effect and a significant improvement in the landscape of red leaves in Beijing. Furthermore, the combination of propiconazole via irrigation plus the trunk injection of carbendazim and prochloraz had the greatest control effect. These suppressive measurements were further used and demonstrated to be effective in Badaling Forest Park. Overall, our study provides an effective disease management means for controlling Verticillium wilt in smoke trees.

Keywords: Verticillium wilt; smoke tree; integrated management; trunk injection; agent irrigation

Citation: Li, B.; Guo, R.; Zhao, Y.; Li, Q.; Song, L.; Shen, C.; Du, C.; Gu, Y.; Qiao, G.; Wang, L.; et al. Development of Integrated Control for Verticillium Wilt of Smoke Trees in Beijing. *Forests* **2024**, *15*, 776. https://doi.org/10.3390/f15050776

Academic Editors: Young-Seuk Park, Won Il Choi and Jong-Kook Jung

Received: 4 April 2024
Revised: 22 April 2024
Accepted: 24 April 2024
Published: 28 April 2024

1. Background

Verticillium dahliae is a widespread and important soil-borne pathogen that infects plants through their roots, colonizing the xylem and causing characteristic wilt symptoms [1]. These symptoms include yellowing, stunting, and necrosis, resulting in billions of dollars of damage per year [2,3]. In China, the pathogen causes diseases in a wide range of plant species, including woody plants [4], flowers [5], cotton [6], and potato [7].

Smoke tree (*Cotinus coggygria*) is an excellent ornamental tree, providing red-colored leaves that beautify the landscape. Distributed in Northern (such as Beijing, Hebei, Shandong, and Shanxi provinces), Southern (such as Hubei and Zhejiang provinces), and Southwestern (Shaanxi Province) China, smoke tree forested areas are an important part of the landscape of red leaves in China and Beijing during autumn [8]. However, the fungus *V. dahliae* causes Verticillium wilt on smoke trees, leading to widespread death and culminating in enormous economic losses. According to the official statistics of Beijing, to date, the total area of smoke trees in Beijing is 251.67 km^2, and the total incidence area of sumac blight is 11.67 km^2, with an incidence rate of 4.63%. In the main areas of smoke trees, such as Xiangshan Park, Badaling Forest Farm, and the National Botanical Garden, the incidence ranges from 36.00 to 62.50%, severely destroying these landscapes. Therefore, it is imperative to develop effective measures to control Verticillium wilt.

Methods for controlling Verticillium wilt have focused mainly on biological and chemical control [9]. Among these, fungicides have been commonly used in the prevention and control of Verticillium wilt, and they can be applied via soil treatment, sprays, and agent irrigation. Soil treatments significantly lowered the incidence of Verticillium wilt in olive trees transplanted into sterilized soil compared to that of those transplanted into untreated soil [10]. Chemical agents spray with two chemical fungicides have been investigated for the control of Verticillium wilt in cotton, and 0.3% tetramycin was shown to slow the spread of the disease and reduce losses [11]. Irrigation water is conducive to the spread of *V. dahliae* microsclerotia, which can increase the incidence and severity of Verticillium wilt [12]. The addition of fungicides to irrigation water can effectively control disease incidence and reduce the survival of *V. dahliae*, sometimes by up to 100% [12,13]. Microbial agents have been extensively researched for biological control in recent years as environmentally friendly alternatives to chemical agents. When *Bacillus velezensis* XT1 was applied to olive seedlings, the incidence rate and severity of wilt were reduced by 54% and 80%, respectively [14]. However, previous studies had problems in terms of their application periods, which were over the course of a year, and low number of applications. The effectiveness of a single application method for Verticillium wilt control has been low, and in the past, irrigation has mostly been used for wilt control. Although this method can eliminate pathogens in the soil and roots, the inhibitory effect on pathogens present in the vascular bundle is limited, and the blockage of vascular bundles may limit transport upward in the tree.

This study aimed to explore an efficient control measure for smoke tree wilt, including efficient application methods and biocontrol agent or fungicide combinations. Experiments were conducted over three consecutive years during the growth period of smoke trees to analyze the effectiveness of agent irrigation, trunk injection, or a combination of these methods for pesticide delivery. Additionally, one microbial agent and four chemical fungicides were evaluated either alone or in combination. The application methods and combinations of agents used provide a basis for controlling Verticillium wilt in smoke trees and potentially other forest trees.

2. Methods

2.1. Experimental Sites' Information

In this study, the experimental sites were located in Xiangshan Park (39°59′24″ N, 116°10′23″ E) and the Hongyeling Scenic Area of Badaling Forest Park (40°25′ N, 116°65′ E), Beijing. The two regions have a temperate semi-humid and semi-arid monsoon climate, with hot, rainy summers and cold, dry winters. During the period of study for the experiments detailed herein, the average annual temperature in Xiangshan Park was 12.5 °C, and the average annual rainfall was 62.89 cm. The average annual temperature in Badaling was 11.8 °C, and the average annual rainfall was 45.74 cm. These two areas have a vast expanse of forest with over 100,000 smoke trees and are representative distribution areas of smoke trees. At the same time, the smoke trees in the experimental sites were planted in mixed clusters with no fixed arrangement, with the distance between each smoke tree varying from 2 to 4 m. Verticillium wilt is a prevalent disease in these regions [8].

2.2. Agent Information and Application Methods

The following agents were used in this study: 100 billion spores/g *B. subtilis* water suspension (biocontrol agent; Hebei Zhongbao Green Crop Technology Co., Ltd., Langfang, China, diluted 1000 times); 50% azoxystrobin (painted green) water-dispersible granules (methoxy acrylate fungicides; Syngenta Agrochemical Co., Ltd., Shenzhen, China, diluted 3000 times during irrigation and 1000 times during trunk injection); 156 g/L propiconazole (green) EC (triazole fungicides; Syngenta Agrochemical Co., Ltd., Shenzhen, China, diluted 3000 times); 50% carbendazim wet table powder (benzimidazole fungicides; Hebei Zhongbao Green Crop Technology Co., Ltd., Langfang, China, diluted 1000 times); and 45% prochloraz aqueous emulsion (imidazole fungicides; Suzhou Fumeishi Plant Protection Agent Co., Ltd., Suzhou, China, diluted 1000 times). The biocontrol agent and fungicides were diluted according to the manufacturer's instructions and recommendations. Meanwhile, these agents have been approved for use by relevant management departments.

For agent irrigation, the biocontrol agent and fungicides (*B. subtilis*, azoxystrobin, or propiconazole) were diluted in 10 L of clean water according to the instructions, and slowly poured onto the soil around the base of the tree trunk and the soil in the tree pit, where the fine roots of the smoke tree were also located. For trunk injection, a tree trunk punching machine (Lvyou Machinery Group Co., Ltd., Beijing, China, model: ZYJ15A) was used to drill a hole at the base of the plant at an angle of 45° from top to bottom, with a depth of about 1/3 of the diameter of the trunk. After punching, an injection port was inserted, and an injection volume of 100 mL (carbendazim and azoxystrobin 1:1 mixture or carbendazim and prochloraz 1:1 mixture) was applied manually to ensure that the agent was injected into the trunk. Once the pressure was reduced, it was removed and sealed with a supporting degradable stopper. The combined application is a combination of agent irrigation and trunk injection. The specific irrigation and injection methods were the same as those used for individual applications.

2.3. Experimental Design

The experiments were conducted from 2021 to 2023. In May 2021, an experiment was conducted based on a completely randomized design. The experiment included 12 treatments, and the environmental conditions were consistent across all treatments (there were footpaths adjacent to the woodland where the sample trees are located, the terrain was flat, and the woodland was free of other tree species but had a weed cover). Each treatment comprised four replicates, with five trees per replicate (i.e., each treatment included 20 smoke trees). A total of 240 smoke trees with the following characteristics were selected: trees aged between 13 and 15 years; with a trunk diameter between 7.3 and 13.6 cm; with an incidence rate of Verticillium wilt that reached 72.08%; and with a disease index that reached 37.6 during this period. The same trees were subjected to the same treatment over the years. The detailed agent application design is shown in Table 1. Treatment 1 was the blank control; treatments 2, 3, and 4 were agent irrigation treatments; treatments 5 and 6 were trunk injection treatments; and the remaining 6 treatments were combined application treatments. The annual application period was from April to October, excluding the rainy season in July. The first application period in 2021 was in May. Agent irrigation was applied four times per year: twice in the period from April to June and twice in the period from August to October, with a one-month interval between applications. The application period and timing for trunk injection and combined application were the same as those for agent irrigation. No additional management measures were implemented for the experimental smoke trees apart from regular irrigation and our applications for the experiments.

Table 1. Experimental design of control measures.

Treatment	Method of Application	Irrigation Agents	Injecting Trunk Agents
1	Blank control		
2	Agent irrigation	*Bacillus subtilis*	
3		azoxystrobin	
4		propiconazole	
5	Injecting trunk		carbendazim, prochloraz
6			carbendazim, azoxystrobin

Table 1. *Cont.*

Treatment	Method of Application	Irrigation Agents	Injecting Trunk Agents
7	Combined treatment	*Bacillus subtilis*	carbendazim, prochloraz
8		*Bacillus subtilis*	carbendazim, azoxystrobin
9		azoxystrobin	carbendazim, prochloraz
10		5azoxystrobin	carbendazim, azoxystrobin
11		propiconazole	carbendazim, prochloraz
12		propiconazole	carbendazim, azoxystrobin

2.4. Evaluation of the Control Effect

According to the grading standard for Verticillium wilt (Table 2) [15], the disease representative values associated with the different treatments were observed monthly from May to October. The disease index for each month and incidence rate were calculated according to the formula in [8]. The relative disease indices of each treatment from June to October were calculated according to the formula using the disease index in May as a baseline value. Subsequently, the preventative and treatment effects of the different agents or application methods were assessed accordingly. For the three application methods, the incidence rate and relative disease index were used to evaluate their effectiveness. The method with the lowest incidence rate and relative disease index was considered the best application method.

$$\text{Incidence rate} = \frac{\text{number of diseased plants}}{\text{total checked plants}} \times 100\%$$

$$\text{Disease index} = \frac{\sum \text{severtiy scale} \times \text{number of diseased plants at this scale}}{\text{total checked plants} \times 4} \times 100$$

$$\text{Relative disease index} = \text{Specific disease index} - \text{Initial disease index}$$

Table 2. Grading standard of Verticillium wilt.

Grade	Grading Criteria	Representative Values
I	Healthy and disease-free plants	0
II	0 < Diseased leaves ≤ 25%	1
III	25% < Diseased leaves ≤ 50%	2
IV	50% < Diseased leaves ≤ 75%	3
V	75% < Diseased leaves ≤ 100%	4

2.5. Disease Index Scale

At the time of the first annual disease investigation (May), the disease severity of smoke trees was divided into three categories according to the disease representative values: healthy (0), mild disease (1–2), and severe disease (3–4) [8]. Using the disease severity data from May as background values, monthly surveys were carried out from June to October to study the changes in and the relative disease index of each category from June to October. At the end of the year (October), a summary was made to determine whether there had been an increase or decrease in disease severity after biocontrol agent or fungicide application to evaluate the preventive or therapeutic effects of different agents and combinations for different disease severities.

2.6. Red Leaf Index

From October to November, the red leaf index of each treatment was observed according to the standard of red smoke tree leaves (Table 3). Three surveys were conducted during the period from the onset of discoloration to leaf loss. The red leaf index of each treatment was calculated according to the following formula:

$$\text{Red leaf index} = \frac{\sum \text{scale} \times \text{number of plants at scale}}{\text{total checked plants} \times 5} \times 100$$

Table 3. Grading standard of red smoke tree leaves.

Grade	Grading Criteria	Representative Values
I	Red leaves ≤ 20%	1
II	20% < Red leaves ≤ 40%	2
III	40% < Red leaves ≤ 60%	3
IV	60% < Red leaves ≤ 80%	4
V	80% < Red leaves	5

2.7. Statistical Analysis

SPSS software (v21.0; SPSS Inc., Chicago, IL, USA) was used to analyze and test the relative disease index data of the treatments. We used the Kruskal–Wallis test as our non-parametric test for the analyses. If the p-value was less than 0.05, it meant that the difference in the data was statistically significant. Then, we performed paired comparison with Bonferroni correction to determine the significance of the differences between treatments (α = 0.05). Figures were created using the ggplot2 package in R (v4.3.2, AT&T Bell Laboratories, University of Auckland, Auckland, New Zealand).

3. Results

3.1. Comparison of the Effects of Different Application Methods

In 2021, the incidence rates for agent irrigation, trunk injection, and the combined application were 93.33, 100, and 84%, respectively. Among them, agent irrigation and the combined application resulted in lower rates compared to the blank control (100%; Table 4), while the incidence rate of trunk injection was the same as that of the blank control. In 2022, the incidence rates for the three application methods were 70, 65, and 65.83%, which were lower than that of the blank control (95%; Table 4). In 2023, the incidence rates for the three application methods were 45, 55, and 40.9%, which were lower than that of the blank control (65%; Table 4). For the relative disease index, the values for agent irrigation, trunk injection, and the combined application were lower than the blank control in all 3 years (Table 4). In 2021, the disease index of the combined application increased by only 7.5, which was lower than those of agent irrigation and trunk injection (18.75 and 14.38, respectively). In 2022, the relative disease indices of the three application methods were 16.44, 18.42, and 15.29, and the index of agent irrigation decreased from the previous year, while the indices of the remaining two increased from the previous year. In 2023, the relative disease indices of the three application methods were 2.75, 4.13, and 2.82, and the values for all three application methods were lower than those in 2021 and 2022 (Table 4). In summary, after three consecutive years of treatment, the combined application showed the lowest incidence rate and relative disease index, followed by agent irrigation, while trunk injection had the lowest effect.

Table 4. Incidence rate and relative disease index of different application methods in three years.

Year	Application Method	Incidence Rate (%)	Relative Disease Index
2021	Blank control	100	31.25
	Agent irrigation	93.33	18.75
	Trunk injection	100	14.38
	Combination	84	7.5

Table 4. *Cont.*

Year	Application Method	Incidence Rate (%)	Relative Disease Index
2022	Blank control	95	28.95
	Agent irrigation	70	16.44
	Trunk injection	65	18.42
	Combination	65.83	15.29
2023	Blank control	65	10.53
	Agent irrigation	45	2.75
	Trunk injection	55	4.13
	Combination	40.9	2.82

3.2. Comparison of the Relative Disease Indices among Treatments

In this study, 12 agent treatments were designed to test their effectiveness in controlling Verticillium wilt. The relative disease index of all the applied treatments was less than that of the blank control (Table 5). For the three agent irrigation treatments, the disease index of the *B. subtilis* irrigation treatment (treatment 2) increased by 17.5 in 2021, which was the same as that of the propiconazole irrigation treatment (treatment 4). The relative disease index of the azoxystrobin irrigation treatment (treatment 3) was the highest (21.25; Table 5). In 2022 and 2023, the disease indices of treatment 2 increased by 9.72 and 1.47, respectively, which were lower than those of treatments 3 (11.66 and 2.94, respectively) and 4 (27.94 and 3.84, respectively). However, there was no significant difference among the three treatments over the three years ($p > 0.05$; Table 5). For the two trunk injection treatments, the disease indices of the trunk injection of carbendazim and azoxystrobin (treatment 6) in 2021 and 2023 increased by 13.75 and 3.95, respectively, which were better than those of the trunk injection of carbendazim and prochloraz (treatment 5). However, in 2022, that of treatment 5 (17.1) was lower than that of treatment 6 (19.74; Table 5).

Table 5. Relative disease index of different treatments in three years.

Treatment	Application Method	Relative Disease Index		
		2021	2022	2023
1	Blank control	31.25 [a]	28.95 [a]	10.53 [a]
2	Agent irrigation	17.5 [abc]	9.72 [ab]	1.47 [ab]
3		21.25 [ab]	11.66 [ab]	2.94 [ab]
4		17.5 [abc]	27.94 [a]	3.84 [ab]
5	Trunk injection	15 [abc]	17.1 [ab]	4.31 [ab]
6		13.75 [bc]	19.74 [ab]	3.95 [ab]
7	Combination	6.25 [bc]	10 [ab]	−0.41 [b]
8		1.25 [c]	13.75 [ab]	1.02 [ab]
9		-	18.33 [ab]	3.33 [ab]
10		15 [abc]	30 [a]	10.94 [a]
11		11.25 [bc]	−1.39 [b]	−1.84 [b]
12		3.75 [c]	21.06 [ab]	3.88 [ab]

[a] In June 2021, due to the severe incidence of Verticillium wilt in smoke trees in treatment 9, the Xiangshan Park Administration undertook large-scale pruning efforts for smoke tree branches within this treatment; therefore, it does not have a reference value. [b] The *p*-values of Kruskal–Wallis test for the years 2021, 2022, and 2023 were 0.002, 0.01, and 0.01, respectively, which were less than 0.05, i.e., the difference in the data was statistically significant. [c] Different letters in the same column of data indicate statistically significant differences between treatments per year ($p < 0.05$; n = 4).

Among the treatments, the disease index of *B. subtilis* irrigation combined with the trunk injection of carbendazim and azoxystrobin (treatment 8) increased by 1.25, which was the lowest in 2021. The index of this treatment was significantly higher than those of treatments 1 and 2 ($p < 0.05$; Table 5). This was followed by treatment 7 (6.25) and treatment 12 (3.75). The relative disease indices of the other treatments were higher than 10 (Table 5). In the next two years, the combination of propiconazole irrigation with the trunk injection of carbendazim and prochloraz (treatment 11) exhibited the most effective control. The disease index decreased by 1.39 in 2022, which was significantly higher than that of treatments 1, 4, and 10 ($p < 0.05$), and decreased by 1.84 in 2023, which was significantly higher than that of treatments 1 and 10 ($p < 0.05$; Table 5). In addition, the relative disease indices of treatment 7 over the three years were 6.25, 10, and −0.41, which were second only to those of treatment 11 out of all the treatments (Table 5). The results of the three-year experiments are shown in Figure 1. In summary, after three consecutive years of treatment, we found that treatment 11 had the most optimal effect on the disease, followed by treatment 7.

Figure 1. Results of a three-year study for control of Verticillium wilt on smoke tree in Xiangshan Park. (**A**–**C**) Comparison of disease occurrence of smoke tree No. 26 (only one) in September 2021 (**A**), September 2022 (**B**), and September 2023 (**C**). (**D**) Red leaf of smoke tree No. 26 (only one) in 2023. (**E**–**G**) Comparison of disease occurrence of smoke tree No. 81 (only one) in September 2021 (**E**), September 2022 (**F**), and September 2023 (**G**). (**H**) Red leaf of smoke tree No. 81 (only one) in 2023.

3.3. Control Effects of Different Treatments on Disease Severity

By comparing the changes in the relative disease index of different disease severities under various treatments from 2021 to 2023, we observed that the trunk injection of carbendazim and azoxystrobin (treatment 6) resulted in the lowest relative disease index in healthy trees in 2021, which was 0. This was followed by the azoxystrobin irrigation treatment (treatment 3) and a combination of *B. subtilis* irrigation with the trunk injection of carbendazim and prochloraz (treatment 7), which yielded values of 25 and 23.31, respectively. The relative disease index of the other treatments was above 30. In 2022, the relative disease index for healthy trees was the lowest in treatment 7, at only 17.31, followed by those of treatments 2, 11, and 12 (20, 19.44, and 19.64, respectively). The relative disease indices of the other treatments were above 20. In 2023, the relative disease index for healthy trees was the lowest in treatments 6 and 11 (both at 0), followed by treatments 2 and 3, which were 2.5 and 4.55, respectively. In addition, compared to the first year, the relative disease index of treatment 2 decreased by 47.5, which was higher than that of the other treatments. The relative disease indices of the other treatments were above 5 (Figure 2A, Table S1). For mildly diseased trees, treatment 7 had the lowest relative disease index in 2021, which was −5, followed by treatments 6 (−2.78) and 9 (0). The relative disease indices of the other treatments were above 0 (Figure 2B, Table S1). In 2022 and 2023, treatment 11 had the greatest effect on trees with mild disease, with relative disease indices of −22.23 and −25, respectively. This was followed by treatments 7 (−4.17 in 2022 and −21.87 in 2023) and 8 (−5.56 in 2022 and −15 in 2023). Additionally, compared to the first year, treatment 11 exhibited the highest reduction in the relative disease index, which was 50 (Figure 2B, Table S1). For severely diseased trees, in 2021, treatment 7 had the lowest relative disease index at −50, followed by treatment 2 at −25. The relative disease indices of the other treatments were above −15 (Figure 2C, Table S1). In 2022, there were no severely affected trees in treatment 11. The relative disease indices of treatments 2, 3, 4, and 9 were 0, while those of the other treatments were above 0 (Figure 2C, Table S1). In 2023, the effects of treatments 7, 8, 9, and 11 were the same, and their relative disease indices were all −25, followed by treatment 10 (−8.33). Those of the other treatments were greater than or equal to 0 (Figure 2C, Table S1). In addition, as was the case with trees with mild disease, treatment 11 showed the greatest reduction in relative disease index compared to the first year, at 31.25. In summary, after 3 years of treatment, treatments 2 and 6 had a better preventative effect against disease for healthy smoke trees. Treatment 11 had the greatest effect on mildly and severely diseased smoke trees, followed by treatment 7.

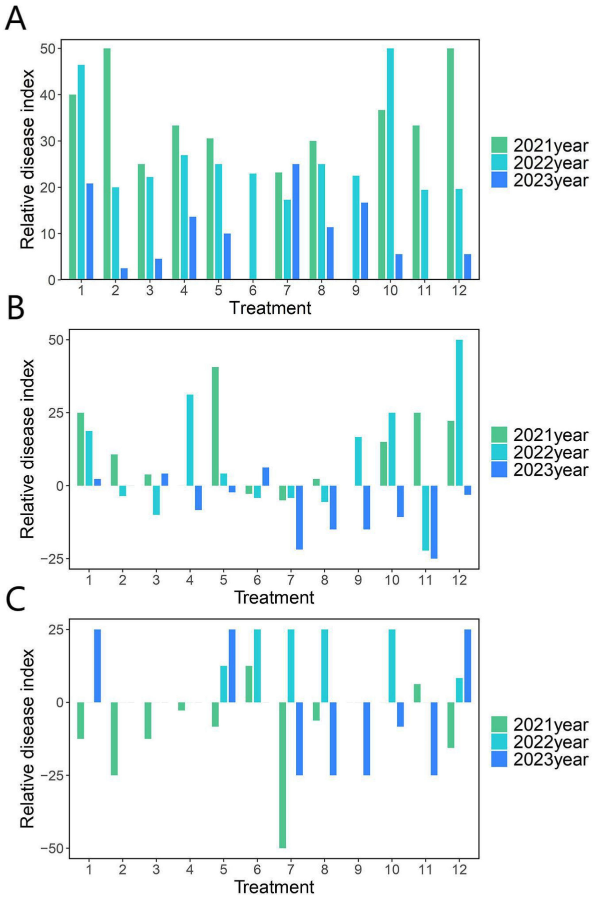

Figure 2. Relative disease index of smoke trees with different degrees of Verticillium wilt from 2021 to 2023. (**A**) Relative disease index of healthy smoke trees. (**B**) Relative disease index of mildly diseased smoke trees. (**C**) Relative disease index of severely diseased smoke trees. In June 2021, due to the severe incidence of Verticillium wilt in the smoke trees in treatment 9, the Xiangshan Park Administration undertook large-scale pruning efforts for smoke tree branches within this treatment, and therefore it does not have a reference value.

3.4. Incidence Rates and Control Effects of Each Treatment by Season

As the annual application period ran from April to October, the incidence rates and relative disease indices of each treatment were calculated and studied in the spring (May–June), summer (July–August), and autumn (September–October) of each year. In October 2021, the incidence rate of each treatment showed different trends, among which the incidence rate of treatment 8 decreased 5% compared to its value in May, treatments 4 and 6 had no increase, and the rest of the treatments showed an increase in incidence (Table S2). In 2022, the incidence rate of each treatment decreased compared to the previous year, among which all the treatments showed an increase in incidence except for treatment 11 which had a 5% decrease in incidence compared to its value in May of the same year (Table S2). The trend of incidence rate changes in 2023 for all the treatments differed from those of the previous two years; it had a decreasing trend and was the lowest out of the three years. Among them, treatment 11 decreased by 25% and performed the best out of all the treatments (Table S2).

In the spring of 2021, the disease index of treatment 12 decreased by 3.75, and the rest of the treatments showed an increase in the disease index. In the summer, the *B. subtilis* irrigation treatment (treatment 2) had the lowest relative disease index (−7.5), followed by treatments 8 and 12 (both at −2.5). In the autumn, treatments 8 and 12 had the lowest relative disease indices of 1.25 and 3.75, respectively. Treatment 7 ranked third with 6.25, and those of the remaining treatments were higher than 10 (Figure 3A, Table S3). In the spring of 2022, a low relative disease index was observed in treatments 3 and 12, at −0.96 and 3.95, respectively. The effectiveness of the other treatments was higher than 4. Treatment 5 exhibited the lowest relative disease index in the summer (2.13), while those of the other treatments were higher than 7. In autumn, the disease index of treatment 11 decreased by 1.39, and those of the rest of the treatments increased and had values higher than 9 (Figure 3B, Table S3). In 2023, treatment 12 had the lowest relative disease index in the spring (3.95), followed by treatments 7 (5.55) and 11 (4.74). The other treatments had relative disease indices less than 6. In the summer, lower relative disease indices were observed in treatments 2, 6, 8, and 9, which reached −1.36, −0.22, −0.36, and −5, respectively. The other treatments had relative disease indices higher than 0. In the autumn, the disease index of treatment 11 decreased by 1.84, followed by treatment 7 (decreased by 0.41). The remaining treatments showed values above 0 (Figure 3C, Table S3). The red leaf index of the application treatments ranged from 31 to 52. Treatments 8 and 12 performed optimally, with 52 and 51.5, respectively, while the lowest value was 31 (treatment 1; Figure 3D and Table S3). In summary, the treatments with the optimal control effect in spring, summer, and autumn were treatment 12, 2, and 11, respectively.

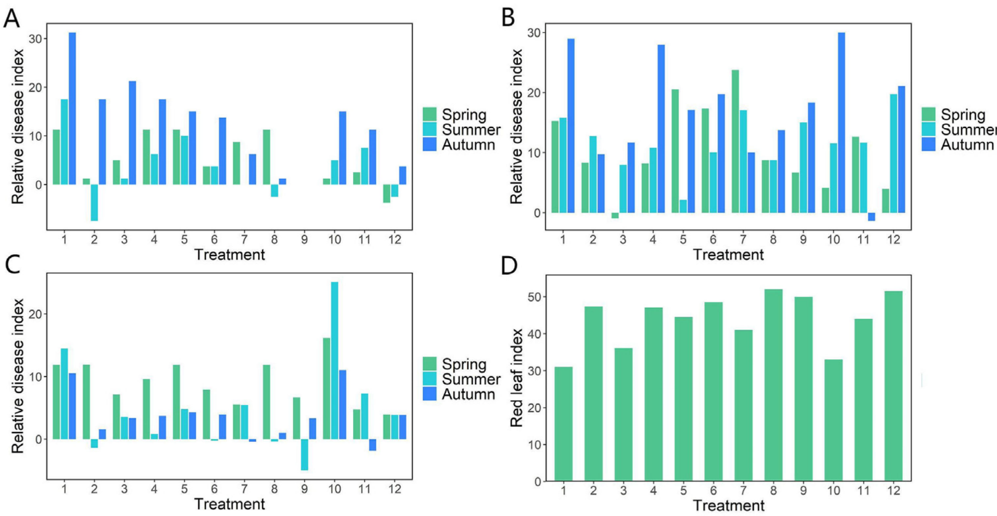

Figure 3. Relative disease indices of each treatment on Verticillium wilt of smoke trees in different seasons within three years and the red leaf index in 2023. (**A**) Relative disease index of each treatment in spring, summer, and autumn 2021. (**B**) Relative disease index of each treatment in spring, summer,

and autumn 2022. (**C**) Relative disease index of each treatment in spring, summer, and autumn 2023. (**D**) Red leaf index of each treatment in 2023. In June 2021, due to the severe incidence of Verticillium wilt in the smoke trees in treatment 9, the Xiangshan Park Administration undertook large-scale pruning efforts for smoke tree branches within this treatment, and therefore it does not have a reference value.

3.5. Verification of the Efficacy of Application Methods and Agents for Badaling Forest Park

We also conducted an identical experiment to control Verticillium wilt in the Hongyeling Scenic Area of Badaling Forest Park. By calculating the differences in the incidence rate, we observed a decrease in the incidence rate in each treatment. The reduction in treatments 2-12 was greater than that in the blank control (treatment 1). Treatment 10 showed the largest reduction in incidence rate, at 77.78%, followed by treatments 7 and 9, at 63.64 and 69.23%, respectively. The incidence rate reduction of the other treatments was less than 60% (Figure 4A, Table S4). The red leaf index increased for all of the treatments except for treatment 3 (with an increase of −6.00) and treatment 6 (with no change in value). Among these, that of treatment 5 was the greatest at 23.95, followed by those of treatments 4, 10, and 12, which were 19.56, 22.23, and 21.33, respectively (Table S4).

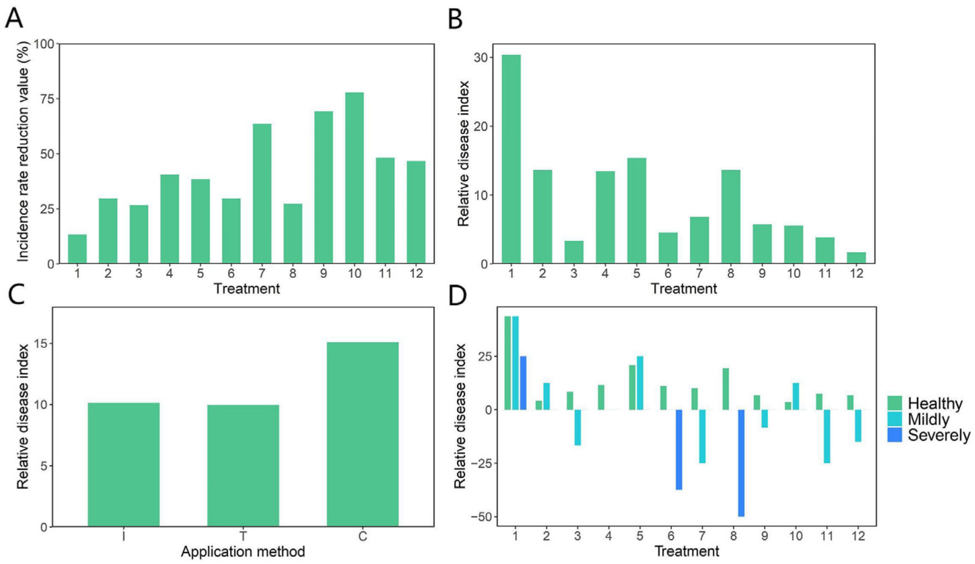

Figure 4. Control effect of Verticillium wilt of smoke trees in Badaling. (**A**) Incidence rate reduction of each treatment from 2021 to 2023. (**B**) Relative disease indices of different treatments in 2023. (**C**) Relative disease indices of different application methods in 2023 (I indicates agent irrigation; T indicates trunk injection; and C indicates combined application). (**D**) Relative disease index of different degrees of disease in 2023.

In terms of the relative disease index of each treatment, treatment 12 had the highest effect, as the disease index increased by only 1.67 in 2023, followed by treatments 3 (3.34) and 11 (3.85) (Figure 4B, Table S5). The calculation of the relative disease indices of the different application methods in 2023 revealed that the combined application had the greatest effect, in which the disease index increased by only 6.22. In comparison, the agent irrigation and trunk injection treatments had relative disease indices of 10.15 and 9.97, respectively (Figure 4C, Table S5). Finally, for the agents targeting different disease severities, the relative disease indices of all the treatments were lower than that of treatment 1 (Figure 4D, Table S6). Among these, treatment 3 had the greatest preventative effect for healthy trees, with a relative disease index of 3.57, followed by treatment 2 (4.17). The relative disease indices of the other treatments were above 6 (Figure 4D, Table S6). Treatments 7 and 11 had the best effect on mild disease, with relative disease indices of −25, followed by treatments 3, 9 and 12 (−16.66, −8.34 and −15, respectively). The remaining treatments yielded values greater than or equal to 0 (Figure 4D, Table S6). In addition, for the severely diseased plants, treatment 8 had the greatest effect with a relative disease index of −50, followed by treatment 6 at −37.5. The relative disease index for the other treatments was 0 (Figure 4D, Table S6). The results of the three-year experiment are shown in Figure 5. Comparing the results of the two regions in 2023, we found that the incidence of disease in Xiangshan Park was milder than that in Badaling Forest Park (Table S7). Among them, the relative disease indices of treatments 3, 6, 9, 11, and 12 showed small differences between the two regions, indicating that these drug treatments have good levels of stability. However, there is a large difference in the relative disease indices of treatments 2, 4, 5, and 8, indicating that different environments have a greater impact on these treatments (Table S7).

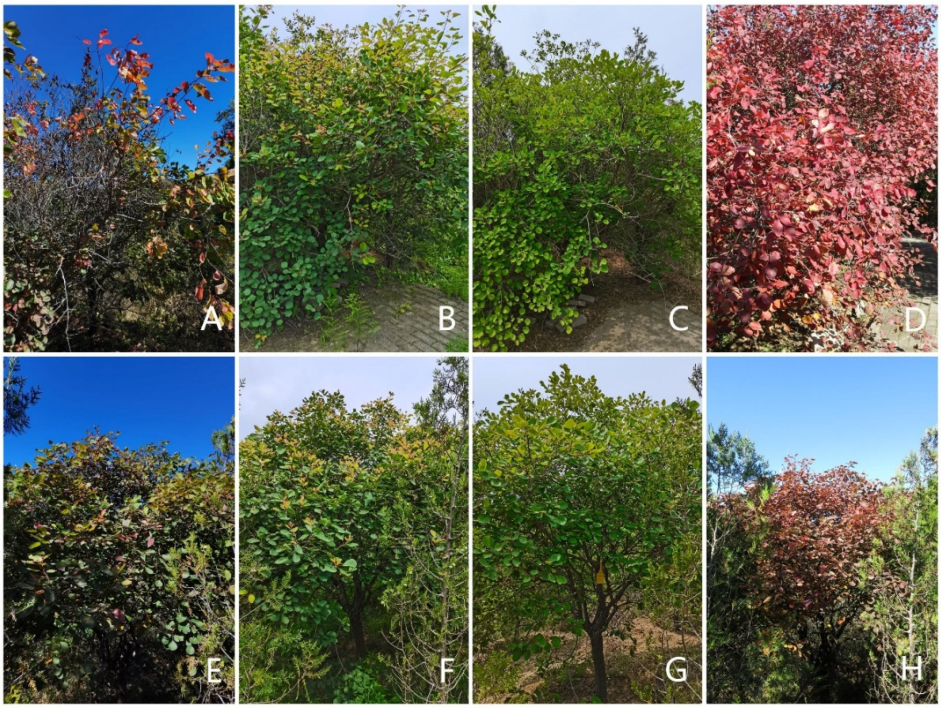

Figure 5. Results of a three-year study for control of Verticillium wilt on smoke tree in Badaling. (**A**–**C**) Comparison of disease occurrence of smoke tree No. 42 (only one) in September 2021 (**A**), September 2022 (**B**), and September 2023 (**C**). (**D**) Red leaf of smoke tree No. 42 (only one) in 2023. (**E**–**G**) Comparison of disease occurrence of smoke tree No. 49 (only one) in September 2021 (**E**), September 2022 (**F**), and September 2023 (**G**). (**H**) Red leaf of smoke tree No. 49 (only one) in 2023.

4. Discussion

Verticillium wilt is difficult to control because of the long-term survival of *V. dahliae* microsclerotia in soil, its broad host range, and the high susceptibility of some plant species [1,2]. In this study, three application methods, namely agent irrigation, trunk injection, and a combination of the two, comprising a biocontrol agent or different fungicides, were evaluated for controlling Verticillium wilt in smoke trees over three consecutive years. The incidence rate and relative disease index of the smoke trees were investigated and calculated to determine the advantages and disadvantages of different application methods as well as the optimum application methods. For three consecutive years, the incidence rate and relative disease index were lowest when agent irrigation was combined with trunk injection. These results were similar to those of the experiment conducted in Badaling Forest Park, indicating that the combined application is an effective application method. However, trunk injection is suitable for large-DBH (diameter at breast height; DBH > 5 cm) trees, and the use of this method can cause mechanical damage to smoke trees with small DBHs (DBH $\leq$ 5 cm), which is not conducive to plant growth. Therefore, in future research, this method will be further optimized to enhance the stability and convenience of trunk injection by reducing the diameter of the drill and improving the injection technology, so as to reduce the possibility of damage to the trees. Additionally, the application range of this technology should be expanded.

With regard to the mechanisms of action of the five agents and their potential impact on the environment, as a growth-promoting bacterium, *B. subtilis* is able to produce VOCs such as salbutamol and 1,3-propanediol, which contribute to plant growth by upregulating photosynthetic activity and phytohormone production [16]. In addition, *B. subtilis* can also produce various lipopeptides (e.g., surfactant, iturin, and fungomycin), cell-cleaving enzymes, antioxidants, and hormones, which have an impact on pathogens and help the plant to resist infection [17]. Meanwhile, abscisic acid (ABA) has been shown to play a key role in ISR induction. *B. subtilis* acts in a similar way to abscisic acid, activating salicylic acid and ABA signaling pathways to close the plant's stomata, thereby limiting pathogen entry into the plant [18]. Moreover, bacterial lipopeptides can also elicit ISR mediated by surfactin and fengycin, which are produced by *B. subtilis* [19]. Carbendazim is a systemic fungicide with both protective and curative activities against a wide range of fungal diseases; this agent can affect cellular mitosis by interfering with spindle formation in the mitosis of pathogenic fungi. However, existing studies have shown that 20%–70% of carbendazim and its degradation products is retained in soil [20]. In the soil environment, carbendazim has adverse effects on animal and micro-organism communities and functions, as well as nutrient biogeochemical cycling [21]. Azoxystrobin is a systemic, broad-spectrum fungicide that acts in the fungal mitochondrion, where it binds to the cytochrome bc complex, preventing electron transport and energy production via oxidative phosphorylation. The residual amount of this fungicide in the soil and plant bodies is very low [22]. Moreover, carbendazim and azoxystrobin have been used in previous studies to control smoke tree wilt, showing good levels of efficacy [8]. Propiconazole is a demethylation inhibitor that has protective, curative, and eradicative activities. Propiconazole interferes with the formation of cell membranes, thus acting as a fungicide, disease protector, and curative agent. However, studies have shown that this agent alters the soil microbial community, leading to a reduction in soil dehydrogenase activity (DHA), which can have adverse effects on soil health and functioning [23]. Prochloraz is a sterol demethylation inhibitor that inhibits sterol biosynthesis, causing the disruption of the cell membrane structure of pathogenic bacteria and ultimately leading to cell death. At the same time, the fungicide prochloraz has been shown to have a negative effect on fungal biomass but not on soil enzyme activity, and it can also be used by bacterial communities as a source of energy and nutrients [24].

As the growth period of smoke trees is from April to October, and pathogenic fungi continue to multiply and infect during this period, we carried out four agent applications in different seasons each year to increase the effectiveness of smoke tree wilt control and protect the health of the trees. Twelve treatments were prepared to evaluate the individual agents or combinations of agents with the best control effect on Verticillium wilt in trees in Xiangshan Park. The same experiment was conducted in Badaling Forest Park to verify the efficacy of the biocontrol agent and fungicides. We found that the incidence rate of each treatment decreased by varying degrees over the three years. For the three irrigation agents, the biocontrol agent *B. subtilis* achieved a relative disease index of 1.58 in 2023, lower than that of azoxystrobin and propiconazole. However, in the Badaling Forest Park experiment, the relative disease index of *B. subtilis* was 13.64. This result may have been influenced by site-specific factors affecting rhizosphere microbiomes [25]. Among the treatments in Xiangshan Park, the combination of propiconazole irrigation with the trunk injection of carbendazim and prochloraz had the lowest relative disease index, which was −1.84 in 2023. This treatment ranked third in the Badaling Forest Park control experiment, suggesting that the control effect of this combination was consistently stable across the sites. In comparison with other studies, there were two main optimizations in our study. First, we combined agent irrigation and trunk injection, rather than limiting them to a single application method. Second, we performed multiple applications throughout the growing season (April–October) for three consecutive years, rather than limiting them to one or two months, which allows us to better understand the long-term control of smoke tree wilt. Therefore, in future research, we recommend using different types or actions of agents for the control of tree wilt through a combination of application methods (not limited to agent irrigation and trunk injection) and extending the application period, which would increase the effectiveness of disease control.

Based on the results of the annual disease survey, the disease incidence of smoke trees was not consistent across the treatments, ranging from mild or even healthy to severe. For these reasons, we analyzed the effectiveness of each agent individually or in combination at different disease levels. The reason that there are three disease severity categories in May is that, first, this is more conducive to graded and precise treatments in subsequent works, as dividing the severity into five levels would make graded treatments too complex and require too much work. Dividing the severity into three levels can maintain high levels of accuracy while reducing researchers' workloads and saving costs. Second, in actual situations in the field, people are more accustomed to distinguishing the degrees of disease in smoke trees as healthy, mildly diseased, and severely diseased. Propiconazole irrigation combined with the trunk injection of carbendazim and prochloraz had an optimal effect on mildly and severely diseased trees. In the Badaling Forest Park control experiment, this treatment was also optimal for mildly diseased smoke trees. *B. subtilis* irrigation (treatment 2) and the trunk injection of carbendazim and azoxystrobin (treatment 6) had better preventative effects when applied to healthy smoke trees. However, in Badaling Forest Park, the effectiveness of treatment 6 was diminished and the relative disease index of treatment 2 was low, second only to that of treatment 10 (Figure 4D, Table S6), indicating that *B. subtilis* exhibited a strong preventive effect against the disease. Based on these results, we surmised that there were differences in the control efficacy of different agents for different degrees of disease. Therefore, it is imperative to distinguish the degree of disease severity and to apply treatments according to the different disease levels. This can also reduce the amounts of agents used, save costs, and decrease environmental pollution while enhancing the efficiency of control measures.

Analyses of the control measures' effects in different periods of the year are important due to the temperature increases in spring during the flowering stage of smoke trees and the early stage of infection with *V. dahliae*. During this period, we found that the combination of propiconazole irrigation with the trunk injection of carbendazim and azoxystrobin yielded the lowest relative disease index. This suggests that this combination was advantageous at that time. In summer, Beijing is characterized by high temperatures and levels of rainfall. These environmental conditions are conducive to the spread of *V. dahliae*. The relative disease index of *B. subtilis* irrigation is the lowest in summer, indicating that using biological agents during this season has the advantage of inhibiting disease spread. Finally, autumn marks the late stage of smoke tree wilt development, during which the wilting and death of smoke trees primarily occur. Propiconazole irrigation combined with the trunk injection of carbendazim and prochloraz had the lowest relative disease index in autumn, indicating that the combination had a desirable effect on late-stage disease control.

This study has three limitations. First, as for the long-term effects of biocontrol agents or fungicides on the environment, as noted above, propiconazole and carbendazim can have adverse environmental effects, and these effects were not previously considered. Second, the two experiment sites in this study were in mountainous regions, and the test smoke trees were mature trees. Thus, the conclusions drawn are generalizable to areas with similar conditions, but under other conditions, such as widely varying temperatures and amounts of rainfall, the range of young smoke trees or other tree species, the incidence of disease, and the efficacy of insecticides may vary, limiting the validity of the conclusions of this study. Third, trunk injection is suitable for trees with a large diameter at breast height (DBH), and the use of this method can cause mechanical damage to smoke trees with a small DBH, which is not conducive to plant growth. In a future study, while investigating the applicability of chemicals and the effectiveness of control measures, we will address the following points to improve upon the current study: reducing or stopping the use of carbendazim and propiconazole; growth and root stimulants to enhance the growth potential and disease resistance of smoke trees; and protocols for the safe, non-destructive management of tree wilt in urban, peri-urban, and other environments. The application method will be further optimized to enhance the stability and convenience of trunk injection by reducing the diameter of the drill and improving the injection technology to reduce the possibility of damage to trees. Additionally, the application range of this technology should be expanded.

5. Conclusions

Our study demonstrated that the combination of agent irrigation and trunk injection treatments had the greatest effect on disease incidence. The combination of propiconazole irrigation and the trunk injection of carbendazim and prochloraz had the greatest effect on the control of the disease in trees in Xiangshan Park for three consecutive years and ranked third in the Badaling Forest Park control experiment. This combination had a lower relative disease index for smoke trees with mild disease in the two regions. Furthermore, *B. subtilis* irrigation had a preventative effect for healthy smoke trees. This study verified the efficacy of controlling Verticillium wilt over many years and assessed the optimal combinations of agents for different situations, thus providing a scientific basis for the control of this disease. According to the guiding ideology of management, more precise control could improve treatment efficiency, reduce the number of inputs, and provide more sustainable and green control measures.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/f15050776/s1, Table S1: Relative disease index of smoke trees with different degrees of Verticillium wilt disease from 2021–2023; Table S2: Incidence rate over the three years; Table S3: Relative disease index of each treatment on Verticillium wilt of smoke trees in different seasons within three years and the red leaf index in 2023; Table S4: Variation of incidence rate and red leaf index of each treatment during 2021–2023 in Badaling; Table S5: Relative disease index of different application methods and different treatments in 2023 in Badaling; Table S6: Relative disease index of different degrees of disease in 2023 in Badaling; Table S7. Comparison of the relative disease index between Xiangshan and Badaling in 2023.

Author Contributions: Y.W. conceived and designed the experiments. R.G., Y.Z., Q.L., L.S., C.S., C.D., Y.G., G.Q., L.W., F.Y. and S.H. participated in this research and collected data. B.L. and Y.W. analyzed the data and wrote the manuscript. B.L. and Y.W. designed the tables and figures. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the National Key Research and Development Program of China (SQ2023XAGG0065) and Municipal Forestry and Parks Resource Conservation Center (2024-ZYBH-02-8).

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Acknowledgments: We would like to thank Beijing Xiangshan Park Administrative Office and Management Department of Beijing Badaling Forest Farm for providing the experiment sites. We would also like to thank Steven J. Klosterman (USDA-ARS, Salinas) for editing the manuscript.

Conflicts of Interest: The authors declare that they have no conflicts of interest.

References

1. Klosterman, S.J.; Atallah, Z.K.; Vallad, G.E.; Subbarao, K.V. Diversity, pathogenicity, and management of *Verticillium* species. *Annu. Rev. Phytopathol.* **2009**, *47*, 39–62. [CrossRef]
2. Chen, J.; Klosterman, S.J.; Hu, X.; Dai, X.; Subbarao, K.V. Key insights and research prospects at the dawn of the population genomics era for *Verticillium dahliae*. *Annu. Rev. Phytopathol.* **2021**, *59*, 31–51. [CrossRef]

3. Inderbitzin, P.; Subbarao, K.V. *Verticillium* systematics and evolution: How confusion impedes Verticillium wilt management and how to resolve it. *Phytopathology* **2014**, *104*, 564–574. [CrossRef] [PubMed]
4. Lu, W.; Liu, Y.; Zhu, H.; Shang, W.; Yang, J.; Hu, X. Verticillium wilt of redbud in China caused by *Verticillium dahliae*. *Plant Dis.* **2013**, *97*, 1513. [CrossRef] [PubMed]
5. Zhang, K.; Jiang, Y.; Zhao, H.; Köllner, T.G.; Chen, S.; Chen, F.; Chen, F. Diverse terpenoids and their associated antifungal properties from roots of different cultivars of chrysanthemum morifoliumramat. *Molecules* **2020**, *25*, 2083. [CrossRef] [PubMed]
6. Xi, H.; Shen, J.; Qu, Z.; Yang, D.; Liu, S.; Nie, X.; Zhu, L. Effects of long-term cotton continuous cropping on soil microbiome. *Sci. Rep.* **2019**, *9*, 18297. [CrossRef] [PubMed]
7. Li, H.; Wang, Z.; Hu, X.; Shang, W.; Shen, R.; Guo, C.; Guo, Q.; Subbarao, K.V. Assessment of resistance in potato cultivars to Verticillium wilt caused by *Verticillium dahliae* and *Verticillium nonalfalfae*. *Plant Dis.* **2019**, *103*, 1357–1362. [CrossRef] [PubMed]
8. Guo, R.; Shen, C.; Li, B.; Du, C.; Li, Q.; Wang, A.; Wang, Y. Chemical control measures of Verticillium wilt in Badaling forest farm. *J. Beijing For. Univ.* **2023**, *45*, 1–9. [CrossRef]
9. Zhang, Y.; Zhao, L.; Feng, Z.; Guo, H.; Feng, H.; Yuan, Y.; Wei, F.; Zhu, H. The role of a new compound micronutrient multifunctional fertilizer against *Verticillium dahliae* on cotton. *Pathogens* **2021**, *10*, 81. [CrossRef] [PubMed]
10. Gomez-Galvez, F.J.; Vega-Macias, V.; Hidalgo-Moya, J.C.; Hidalgo-Moya, J.J.; Rodriguez-Jurado, D. Application to soil of disinfectants through irrigation reduces *Verticillium dahliae* in the soil and Verticillium wilt of olive. *Plant Pathol.* **2020**, *69*, 272–283. [CrossRef]
11. Li, H.; Jin, G.; Cai, X.; Zhang, K.; Chen, Q.; Gu, A. Comparison of the control effects of two kinds of fungicides on cotton Verticillium wilt. *Plant Prot.* **2018**, *44*, 225–229. [CrossRef]
12. Gómez-Gálvez, F.J.; Moya, J.C.H.; Vega-Macías, V.; Hidalgo-Moya, J.J.; Rodríguez-Jurado, D. Reduced introduction of *Verticillium dahliae* through irrigation systems and accumulation in soil by injection of peroxygen-based disinfectants. *Plant Pathol.* **2018**, *68*, 116–126. [CrossRef]
13. Santos-Rufo, A.; Rodríguez-Jurado, D. Evaluation of chemical disinfestants in reducing *Verticillium dahliae* conidia in irrigation water. *Crop Prot.* **2016**, *79*, 105–116. [CrossRef]
14. Castro, D.; Torres, M.; Sampedro, I.; Martínez-Checa, F.; Torres, B.; Béjar, V. Biological control of Verticillium wilt on olive trees by the salt-tolerant strain *Bacillus velezensis* XT1. *Microorganisms* **2020**, *8*, 1080. [CrossRef] [PubMed]
15. Tao, J.; Cui, J.; Sun, T.; Zeng, J.; Su, X.; Zhang, X. Screening of chemicals for control of Verticillium wilt of *Cotinus coggygria* and the effect of adjuvant. *For. Pest Dis.* **2024**, *43*, 28–33. [CrossRef]
16. Tahir, H.A.; Gu, Q.; Wu, H.; Raza, W.; Hanif, A.; Wu, L.; Colman, M.V.; Gao, X. Plant growth promotion by volatile organic compounds produced by *Bacillus subtilis* SYST2. *Front. Microbiol.* **2017**, *8*, 171. [CrossRef] [PubMed]
17. Mahapatra, S.; Yadav, R.; Ramakrishna, W. *Bacillus subtilis* impact on plant growth, soil health and environment: Dr. Jekyll and Mr. Hyde. *J. Appl. Microbiol.* **2022**, *132*, 3543–3562. [CrossRef] [PubMed]
18. Kumar, A.S.; Lakshmanan, V.; Caplan, J.L.; Powell, D.; Czymmek, K.J.; Levia, D.F.; Bais, H.P. Rhizobacteria *Bacillus subtilis* restricts foliar pathogen entry through stomata. *Plant J. Cell Mol. Biol.* **2012**, *72*, 694–706. [CrossRef]
19. Ongena, M.; Jourdan, E.; Adam, A.; Paquot, M.; Brans, A.; Joris, B.; Arpigny, J.L.; Thonart, P. Surfactin and fengycin lipopeptides of *Bacillus subtilis* as elicitors of induced systemic resistance in plants. *Environ. Microbiol.* **2007**, *9*, 1084–1090. [CrossRef] [PubMed]
20. Arya, R.; Sharma, R.; Malhotra, M.; Kumar, V.; Sharma, A.K. Biodegradation aspects of carbendazim and sulfosulfuron: Trends, scope and relevance. *Curr. Med. Chem.* **2014**, *22*, 1147–1155. [CrossRef]
21. Mana, L.; Pinga, L.I.; Zu-Cong, C.J.A. Degradation of carbendazim in soil and its eco-environment effect. *Agrochemicals* **2012**, *51*, 8–11.
22. Joseph, R.S.I. Metabolism of azoxystrobin in plants and animals. *Biol. Environ. Sci.* **1999**, *233*, 265–278. [CrossRef]
23. Sliti, A.; Singh, V.; Ibal, J.C.; Jeong, M.; Shin, J.H. Impact of propiconazole fungicide on soil microbiome (bacterial and fungal) diversity, functional profile, and associated dehydrogenase activity. *Environ. Sci. Pollut. Res. Int.* **2024**, *31*, 8240–8253. [CrossRef] [PubMed]
24. Tejada, M.; Gómez, I.; García-Martínez, A.M.; Osta, P.; Parrado, J. Effects of prochloraz fungicide on soil enzymatic activities and bacterial communities. *Ecotoxicol. Environ. Saf.* **2011**, *74*, 1708–1714. [CrossRef] [PubMed]
25. Kusstatscher, P.; Wicaksono, W.A.; Thenappan, D.P.; Adam, E.; Berg, G. Microbiome management by biological and chemical treatments in maize is linked to plant health. *Microorganisms* **2020**, *8*, 1506. [CrossRef]