agriculture

MDPI

Article

Technology for Distribution and Control of *Agrobacterium tumefaciens* in Cherry Tree Soil

Shenyan Liu [1,2], Yiwen Zheng [3], Kunpeng Yu [3], Shimeng Gao [2], Xiaojuan Zhao [4], Aocheng Cao [2,*] and Qingli Han [1,*]

[1] College of Forestry, Southwest Forestry University, Kunming 650224, China; liushenyan@swfu.edu.cn
[2] State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing 100193, China; shimeng_gao_1019@163.com
[3] College of Plant Protection, Jilin Agricultural University, Changchun 130118, China; ywenzh@outlook.com (Y.Z.); yvkunpeng@outlook.com (K.Y.)
[4] Yuyuantan Park, Beijing 100142, China; zhaoxj86@126.com
* Correspondence: aochengcao@ippcaas.cn (A.C.); hanqingli@swfu.edu.cn (Q.H.)

Abstract: Sakura crown gall, caused by the invasion of *Agrobacterium tumefaciens* through plant wounds, poses a significant threat to cherry trees. In this study, the distribution of *A. tumefaciens* was preliminarily determined by stratified sampling and qPCR detection. Vertically, the pathogen is mainly distributed in the soil layer below 20 cm, and the amount of bacteria increases at greater depths. Horizontally, they are found within a 150 cm radius from the trunk. Zhongshengmycin and Oligosaccharide–Ethylicin were applied 100 cm from the trunk at a depth of below 20 cm. In the 20~40 cm soil layer, a 600-fold diluted solution of 3% Zhongshengmycin had a relative control efficacy of 94% to 100% against *Agrobacterium tumefaciens*, while a 1000-fold diluted solution of 25% Oligosaccharide–Ethylicin showed a control effect ranging from 54% to 100%. Before transplantation, the soil was disinfected with dazomet and abamectin. Application rates were 35 g/m^2 for dazomet (98% granules) and 1 mL/m^2 for abamectin (1.8% emulsifiable concentrate). The disinfection effectiveness was 77~100% in the 0~60 cm soil layer.

Keywords: crown gall; cherry tree; *Agrobacterium tumefaciens*; qPCR detection; soil fumigation; control efficiency; soil pathogen management

Citation: Liu, S.; Zheng, Y.; Yu, K.; Gao, S.; Zhao, X.; Cao, A.; Han, Q. Technology for Distribution and Control of *Agrobacterium tumefaciens* in Cherry Tree Soil. *Agriculture* **2024**, *14*, 1857. https://doi.org/10.3390/agriculture14111857

Academic Editor: Alessandro Vitale

Received: 27 September 2024
Revised: 13 October 2024
Accepted: 15 October 2024
Published: 22 October 2024

1. Introduction

The cherry blossom (*Cerasus* spp.) is a deciduous tree of the rose family (Rosaceae), renowned for its high economic and ornamental value. Cherry trees are picky with their growth environment, and planting them in large numbers over extended periods can exacerbate the occurrence of diseases. Among these, cherry crown gall poses the biggest threat to plant health [1]. Crown gall is a bacterial disease caused by *Agrobacterium tumefaciens*. In 1907, Smith and Townsend first found and named the pathogen [2]. *A. tumefaciens* is a latent soil bacterium capable of causing systemic infection. It usually invades the host plant through wounds and leads to the formation of tumors. *A. tumefaciens* is known to infect 643 plant species across 93 families and 331 genera, among which fruit trees such as peach, pear, cherry, and apple are particularly seriously infected [3]. Crown gall bacteria can overwinter in the cortex of tumor tissue and, when the tumor ruptures, can survive in the soil for more than 1 year. Transmission is usually through rain, irrigation water, underground pests, and many farming tools. Long-distance transmission is commonly facilitated by the transportation of soil, seedlings, and scions through human activities [4].

The impact of crown gall disease is widespread and severe. According to the European and Mediterranean Plant Protection Organization (EPPO) Data Ban, the distribution of plant root cancer has been documented in several countries and regions around the world. In the United States, economic losses from crown gall exceed USD 20 million annually [5]. In China, crown gall has been reported in multiple regions, including Jiangsu, Zhejiang, Fujian,

Hunan, Shanghai, Shandong, Hebei, Dalian, Inner Mongolia, Liaoning, and Beijing. The infection rate among susceptible varieties ranges from 30% to 100%, with a production loss of 30%, and the incidence rate is rising year by year [6–9]. Therefore, the finding of effective prevention and control measures is an urgent need for agriculture and forestry development.

Current research mainly focuses on the pathogenic mechanism and genetic engineering of transgenic plants. Lai and Kado identified the pathogenic mechanism of *Agrobacterium radiculosa* [10], while Lee, Chilton, and Suzuki studied the structure and tumorigenic mechanism of the Agrobacterium Ti plasmid, applying it as a vector in genetic engineering to create transgenic plants [11–13]. However, the emphasis on *Agrobacterium* as a tool for genetic engineering has often overshadowed the study of it as a plant pathogen and of its behavior in the natural environment and in production settings. For instance, so far, the spatial distribution of *A. tumefaciens* in soil has not been reported. In this study, we determined the distribution and abundance of *A. tumefaciens* in soil using absolute fluorescence quantification methods. At the same time, soil fumigation was used to disinfect the replanted soil, eliminate the remaining rhizobacterium in the soil, and initially explore technology for the prevention and control of sakura crown gall.

2. Materials and Methods

2.1. Experimental Materials and Reagents

2.1.1. Bacterial Strains

A standard *A. tumefaciens* strain from the culture center (BN-1) and a previously known isolate from Yuyuantan Park (YY-1) were used as reference strains in this experiment (Table 1).

Table 1. Strains tested.

Bacterial Strain	Source	Code Name
A. tumefaciens standard strain	BNA Biological Strain Preservation Center	BN-1
A. tumefaciens	Soil in Beijing	YY-1

2.1.2. Test Kit

The DNA in this experiment was processed with the kits below (Table 2).

Table 2. Main kits.

Kit	Name	Manufacturer
Bacterial Genomic DNA Purification Kit (including RnaseA)	EasyPure Bacteria Genomic DNA Kit	TRAN (Beijing, China)
Fluorescence dye	SsoFast EvaGreen Supermix	BIO-RAD (USA)
Plasmid mini extraction kit	EasyPure Plasmid MiniPrep Kit	TRAN (Beijing, China)
PCR Product Purification Kit	FastPure Gel DNA Extraction Mini Kit	Novizan (Nanjing, China)
Soil genomic DNA purification kit	FastDNA TM SPIN Kit for Soil	MP (USA)
T1 Gene Cloning Kit (dual antibodies)	pEASY-T1 Cloning Kit	TRAN (Beijing, China)

2.2. Methods

2.2.1. Quantitative Primer Screening

Based on the relevant literature on *A. tumefaciens*, seven potential pairs of specific primers were selected for PCR amplification using BN-1 and YY-1 standard strains [14], as shown in Table 3. Among these, the most effective primer pair was chosen for the quantitative detection of *A. tumefaciens* in cherry trees in Yuyuantan Park, Beijing.

Table 3. Experimental primers and conditions.

Primer Name	Base Sequence (5′to 3′)	Annealing Temperature and Cycle Number	Target Gene Size (bp)	Reference Literature
B1R8	CGATAAAGGGAGTGAGAGACTCC	60 °C 36 cycles	178	Feng Zekun [14]
B1F8	CCAGTAAGAATGTACAACACACGA			
virD2.F	TTGGAATATCTGTCCCGGAAG	60 °C 36 cycles	224	Yakabe and Parker [12]
virD2.R	CTTGTACCAGCAGGGAAGCTTA			
tms 2F1	TTTCAGCTGCTAGGGCCACATCAG	60 °C 36 cycles	220	Tan and Yabuki [15]
tms 2R2	TCGCCATGGAAACGCCGGAGTAGG			
ipt 3F	CGGACGACCAACAGTGAAGA	50 °C 35 cycles	247	Haas and Moore [16]
ipt 3R	TTCGGGTAACTTGTGGCGAA			
F primer	TCCTACGGGAGGCAGCAGT	50 °C 35 cycles	466	Weisburg [17]
R primer	GGACTACCAGGGTATCTAATCCTGTT			
Aar F	GTTACGAGCTGATGATGGAAAGC	53 °C 35 cycles	579	Shi Jinqiao [18]
Aar R	CACCATCCTTCTTCATAATCGCG			
Afa F	GTCAACACCATGCTGGATATGAG	55.9 °C 40 cycles	505	Shi Jinqiao [18]
Afa R	CAGGCAACATGATCGTTACGAC			
CYT	GATCGGGTCCAATGCTGT	55 °C 35cycles	427	Barker and Idler [19]
CYT	GATATCCATCGATCTCTT			

2.2.2. Quantitative PCR of Soil Bacteria

1.2.1 after selecting the appropriate primers, conventional PCR amplification was performed, and PCR products were purified using a Novizan product purification kit. The products were then ligated and transformed using the full gold connection transformation kit, and single clones were selected for liquid sequencing (Genomics, Beijing, China). Plasmid extraction was carried out using the full gold plasmid miniprep kit. A standard curve was then prepared. Soil DNA was extracted using the MP soil genomic DNA purification kit, and the resulting DNA from the soil was used as a template for qPCR [20].

2.3. Field Experiments

2.3.1. Overview of the Experimental Site

The experimental site was Yuyuantan Park in Beijing, China (39.92° N, 116.32° E). Four cherry gardens where crown gall had been reported were selected for this study: the Great Lawn, Li Ying Garden, Wang Ying Garden, and the Friendship Cherry Forest.

2.3.2. Experimental Agents

For the fumigation of replanting pits, the amount of chemical used was calculated according to the recommended rates and planting area. For trees in the growing period, a total of 200 mL of the prepared treatment solution was applied to each tree. The concentration of the treatment solution was determined based on the results from earlier laboratory bioassays. The actual amounts and concentrations used are shown in Table 4.

Table 4. Dosage and field application method.

Chemical Used	Dosage	Use	Manufacturer	Sampling Point Names
1.8% avermectin emulsion	1 mL/m^2	spray	Shandong Yijia Agrochemical Co., Ltd. (Jinan, China)	1 (Great Lawn), 11 (Wan Ying Garden), 17 (Li Ying Garden)
98% dazomet granules	35 g/m^2	soil incorporation	Nantong Shizhuang Chemical Co., Ltd. (Nantong, China)	
25% oligosaccharide—ethylicin microemulsion	in a 1:1000 dilution	root drenching	Hainan Zhengye Biotechnology Co., Ltd.	1, 2, 3
3% zhongshengmycin	in a 1:600 dilution	root drenching	Jiangsu Wanyuan Biotechnology Co., Ltd.	4, 5, 6

2.3.3. Experimental Methods

Soil disinfection was performed on soil after diseased trees were removed and prior to replanting. A pit measuring 200 cm × 150 cm × 60 cm was made. The treatment was applied in layers at depths of 40–60 cm, 20–40 cm, and 0–20 cm using a layered application method. First, an abamectin suspension was sprayed evenly into the 40–60 cm soil layer using a MATABI-16 backpack sprayer. Then, Dazomet was evenly spread on the soil surface. The soil was then thoroughly mixed to ensure a uniform distribution of the chemicals. This operation was repeated for the 20~40 cm and 0~20 cm soil layers [21]. After the layered application, water was poured on the surface of the soil to achieve around 80% field water capacity. The soil was covered with PE film for fumigation for 1 month. The experiment took place in early October, when the temperature in Beijing was around 25 °C, which was more suitable. After film removal, the soil was sampled and brought back to the laboratory and stored in a −80 °C refrigerator for subsequent testing.

For the cherry trees that were still growing and not planned for removal, trees with weak growth and suspected crown gall were selected for root drenching. A circle with a 100 cm radius was drawn around each tree. Four holes, each 20 cm deep, were made along the circle. A total of 200 mL of the prepared treatment solution, featuring either Oligosaccharide–Ethylicin or zhongshengmycin, was applied to each tree, with 50 mL

poured into each hole. The holes were then covered with soil. After 50 days, the soil in the circle was sampled at specific distances and depths around the tree and brought back to the laboratory for testing. Three soil cores were extracted with a soil sampler at distances of 50, 100, and 150 cm from the tree trunk, resulting in a total of nine soil cores per tree. These 60 cm long soil cores were then split into three segments based on depth from the surface (0–20 cm, 20–40 cm, and 40–60 cm). The resulting three samples at each specific distance and depth combination were thoroughly mixed to form one homogeneous composite sample, so that for each tree, there were nine composite samples.

2.4. Data Statistics

The control efficacy against *A. tumefaciens* was calculated using the following formula:

Control efficacy (%) = (Bacterial load before treatment-Bacterial load after treatment)/Bacterial load before treatment × 100%

The test data were organized using Microsoft Excel 2010, and statistical analysis (ANOVA) was performed with SPSS 20.0. Fisher's LSD test (p = 0.05) was applied for comparisons between group means, the percentage data was converted by arcsine and the corresponding Angle value p was obtained, and figures were generated using Origin 2017.

3. Results

3.1. Screening of Specific Primers for A. tumefaciens

The universal primer pair (F primer/R primer) and specific primers (B1R8/B1F8, virD2.F/virD2.R, tms 2F1/tms 2R2, ipt 3F/ipt 3R, Aar F/Aar R, AfaF/Afa R, and CYT/CYT) were tested with conventional PCR with BN-1 and YY-1 as templates, and then verified through gel electrophoresis. Figure 1 shows the results of the gel electrophoresis of eight primer pairs. The specificity of each primer is indicated by the presence of a single, clear band; a strong, bright single band suggests high specificity. In Figure 1, the universal primer showed a single bright band at 466 bp. Among the specific primers, only B1R8/B1F8 showed a consistent, bright, and singular band. Therefore, the B1R8/B1F8 primer was selected for the subsequent qPCR fluorescence quantification, while the other primers required further optimization due to their weak specificity.

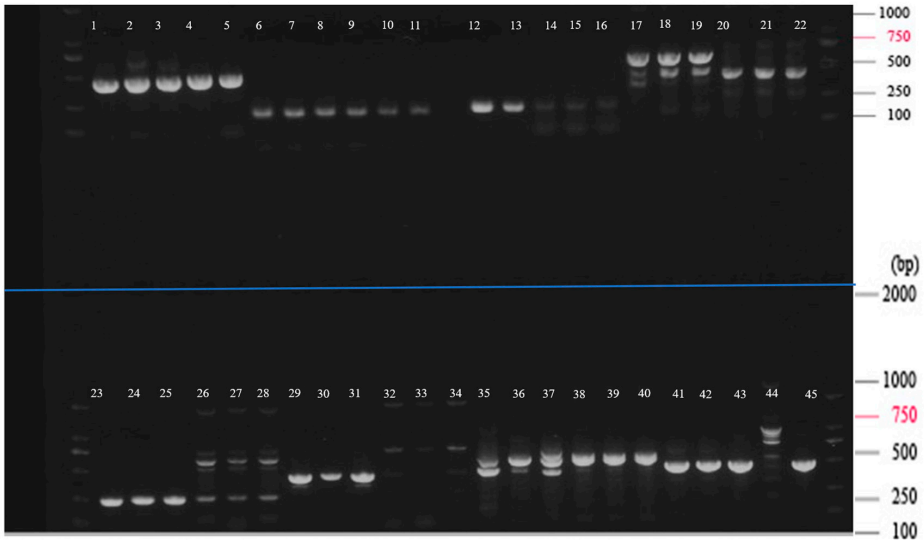

Figure 1. Specific primer screening. Note: F/R primer combinations are as follows: BN-1 (wells 1–2), YY-1 (wells 3–5), specific primer B1R8/B1F8 (wells 6–11), virD2.F/virD2.R (wells 12–13 for BN-1, wells 14–16 for YY-1), tms2F1/tms2R2 (wells 17–22), ipt3F/ipt3R (wells 23–28), AarF/AarR (wells 29–34), AfaF/AfaR (wells 35–40), and CYT/CYT (wells 41–43 for BN-1, wells 44–45 for YY-1). For each primer pair, 5–6 wells were used. The left 3 wells show the BN-1 standard strain, while the right 3 wells display the isolated YY-1 strain.

3.2. Quantitative Calibration Detection of Absolute Fluorescence

In Section 2.1, the primers B1F8/B1R8 showed strong specificity. The qPCR calibration curve, at the annealing temperature of 52 °C and with 38 cycles, is shown in Figures 2–4; when the amplification curve reaches the plateau at the expected cycle number, the dissolution curve shows a single peak, the amplification efficiency is close to 100%, and the R2 value is above 0.9, the specific primers can be used for qPCR detection of specific strains under these conditions. As shown, the specific primers B1R8/B1F8 meet the requirements for real-time fluorescence quantification and can be used for the absolute quantification of *Agrobacterium tumefaciens* in the soil of Yuyuantan Park.

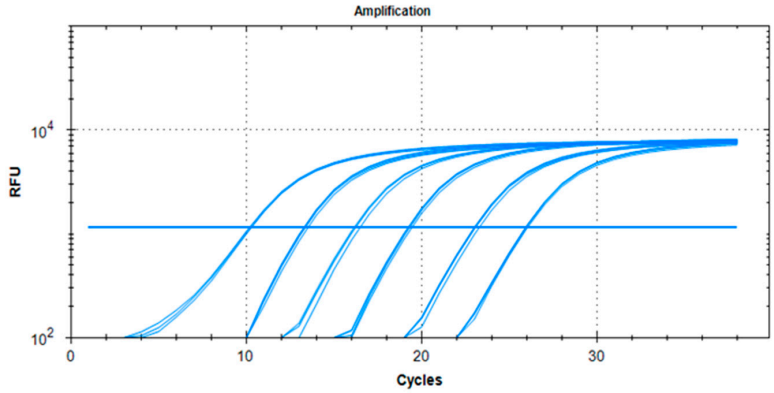

Figure 2. Amplification curve of primer B1R8/B1F8.

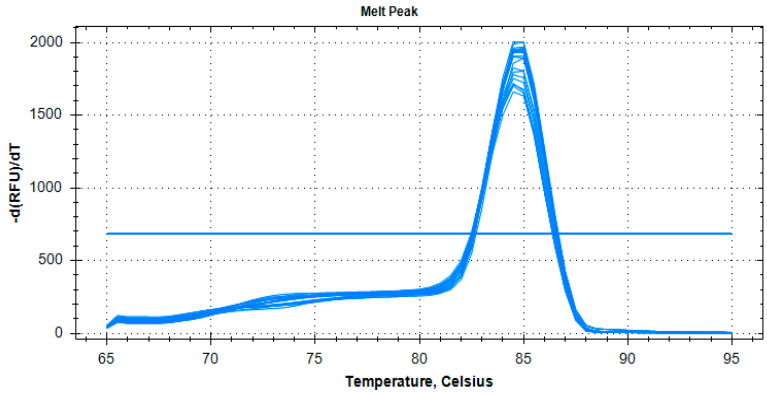

Figure 3. Dissolution curve of primer B1R8/B1F8.

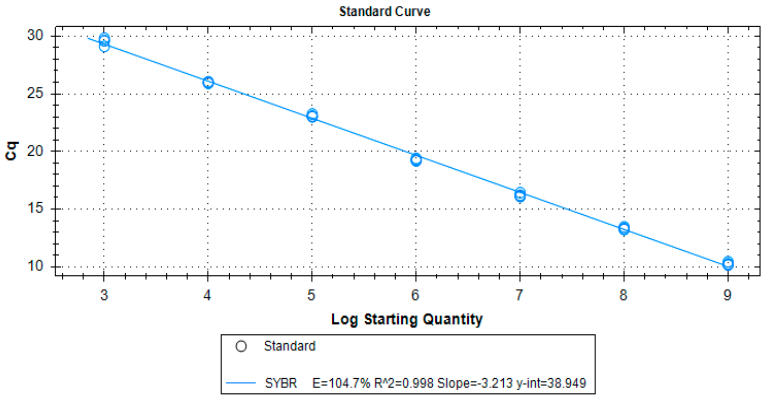

Figure 4. Amplification equation of primer B1R8/B1F8.

3.3. Distribution of Agrobacterium tumefaciens in Cherry Tree Root Soil

Soil DNA was extracted and amplified using conventional PCR with B1R8/B1F8 primers, following analysis by agarose gel electrophoresis to determine the distribution of

A. tumefaciens in the soil. Figure 5 shows the gel electrophoresis results of some samples. Out of the 37 samples analyzed, a single band of varied brightness appeared at 178 bp. Samples 4 to 27 and 38 to 40, taken from soil layers deeper than 20 cm, showed bright bands. In contrast, soil samples taken from 0–20 cm showed no bands. The gel electrophoresis diagram suggests that *A. tumefaciens* was either absent or present at levels undetectable through conventional PCR in the 0–20 cm soil layer. The amount of *A. tumefaciens* in the soil layers below 20 cm should be further quantified.

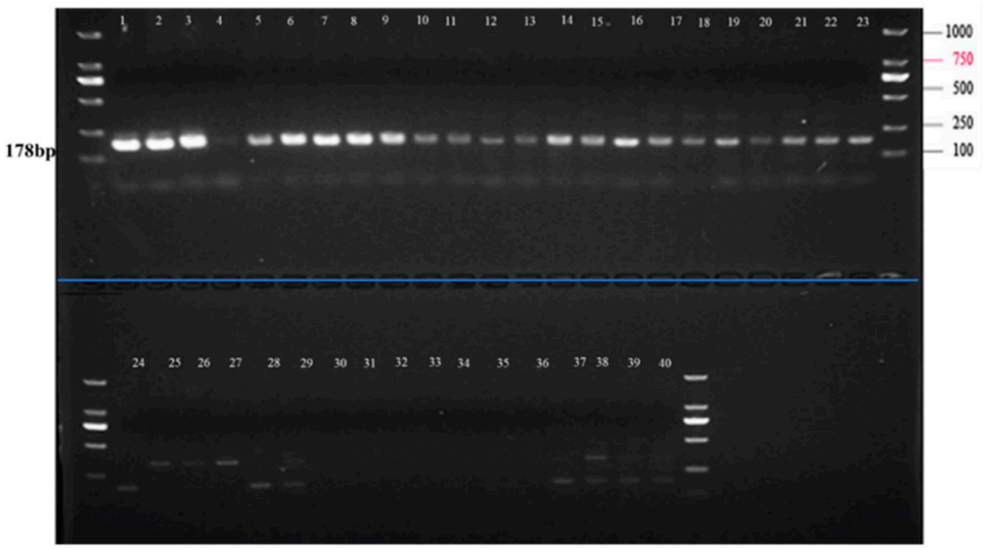

Figure 5. Gel electrophoresis of some samples. Note: 1–3 is the YY-1 strain and 4–40 are soil samples from different locations in Yuyuantan Park.

The amount of *A. tumefaciens* in the soil was further quantified using absolute fluorescence quantification, as shown in Figure 6. Vertically, no *A. tumefaciens* was detected in the 0–20 cm soil layer. In the 20–40 cm soil layer, *A. tumefaciens* was detected in all samples except for two: sampling point 2 at 50 cm and sampling point 6 at 150 cm. In the 40~60 cm soil layer, *A. tumefaciens* was detected at all sampling points. The bacterial load ranged from 10,000 to 90,000 cfu/g. The highest bacterial load was observed at a 100 cm distance in the 40–60 cm soil layer, reaching 867,200 cfu/g. The minimum detected load among the samples was found at a 150 cm distance in the 20–40 cm soil layer, which was 782 cfu/g. No samples were taken at sampling points 4, 5, or 6 in the 50- (40–60) cm range.

Figure 6 shows the bacterial load at different sampling locations. In terms of vertical distribution, the soil layer above 20 cm contained no *Agrobacterium tumefaciens*. It was mainly distributed in the soil layer below 20 cm, and the bacterial load increased with soil depth. Horizontally, the bacterial load did not exhibit a consistent pattern across different distances in the same soil layer.

3.4. The Inhibitory Effect of Dazomet Fumigation on Agrobacterium tumefaciens

The inhibition effects of fumigation with 35 g/m^2 dazomet and 1 mL/m^2 abamectin are shown in Table 5. After soil fumigation, the relative control efficacy against *A. tumefaciens* in the 0~20 cm and 20~40 cm soil layer was 97% to 100%. The relative control effect at the Great Lawn and the Li Ying Garden in the 40~60 cm soil layer was more than 90%. The control efficacy at the Wan Ying Garden was slightly lower, with a relative control effect of 79.28%. This reduced efficacy was likely due to the high number of stones and the large soil porosity in the Wan Ying Garden, which allowed gas to dissipate easily, thereby diminishing the fumigation effect.

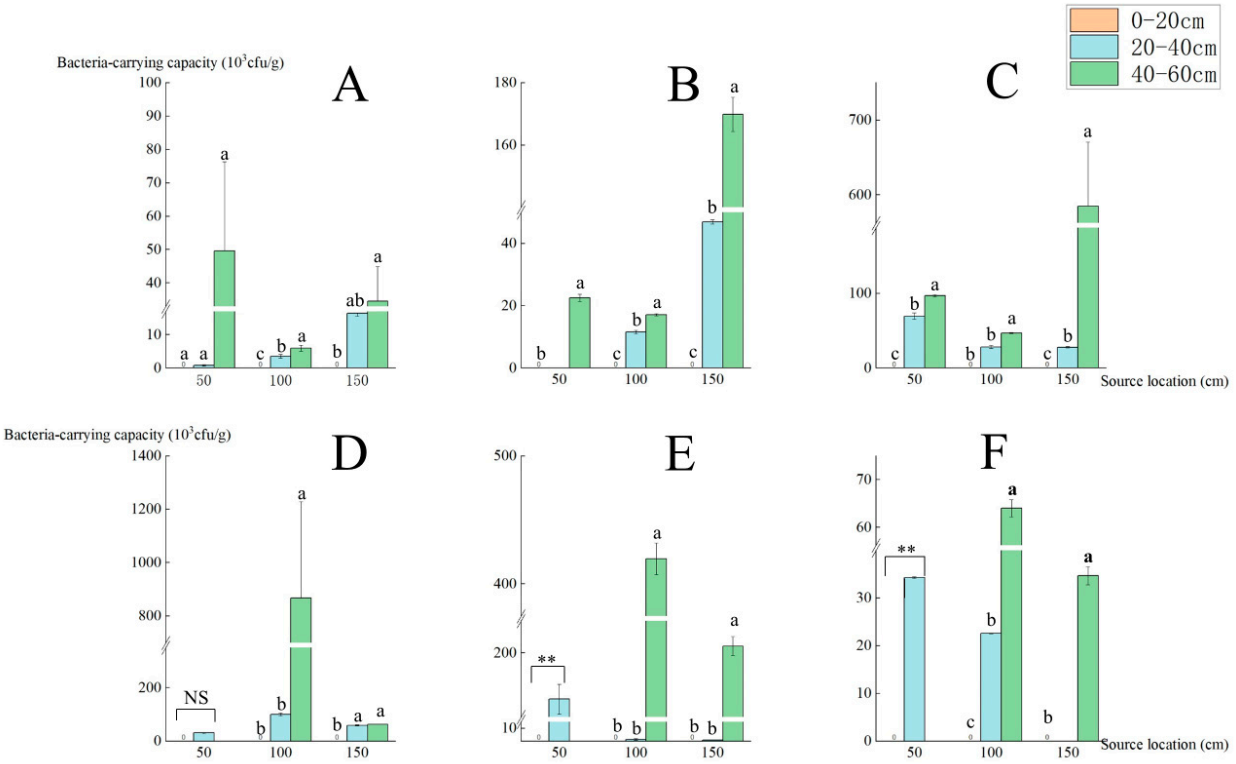

Figure 6. Soil bacterium distribution map. Note: (**A**–**F**) represent the six sampling points; 50, 100, and 150 represent the distance from the tree trunk; and the different colors represent the distance from the ground in centimeters. Different lowercase letters indicate significant differences between treatments ($p < 0.05$); ** represents $p < 0.01$, NS means the difference is not significant.

Table 5. Prevention and treatment effects of dazomet + abamectin on *Agrobacterium tumefaciens*.

Sampling Site	Sampling Depth (cm)	Bacterial Load Before Treatment (1×10^3 cfu/g)	Bacterial Load Post-Treatment (1×10^3 cfu/g)	Relative Efficacy (%)
1	0–20	0	0	100 a
	20–40	10.89 ± 0.65	0	100 a
	40–60	28.00 ± 0.145	0	100 a
11	0–20	0	0	100 a
	20–40	17.56 ± 0.86	0.38 ± 0.15	97.84 ± 0.87 a
	40–60	20.17 ± 2.81	4.18 ± 1.81	79.28 ± 6.54 b
17	0–20	0	0	100 a
	20–40	1.36 ± 0.13	0	100 a
	40–60	5.52 ± 0.43	0.47 ± 0.11	91.54 ± 1.57 b

Different lowercase letters indicate significant differences between different treatments ($p < 0.05$).

3.5. Effect of Root Drenching on Controlling Agrobacterium tumefaciens

The effect of 25% Oligosaccharide–Ethylicin (in a 1:1000 dilution) and 3% zhongshengmycin (in a 1:600 dilution) on the control of *A. tumefaciens* is shown in Figure 7. Zhongshengmycin demonstrated good control efficacy against *A. tumefaciens* in the 20~40 cm soil layer; however, its efficacy in the 40~60 cm layer was lower, likely because the agent did not sufficiently diffuse into that deeper layer. The relative control effect of 25% Oligosaccharide–Ethylicin (1000 times dilution) exceeded 50% in the 20–40 cm soil layer, but in this experiment, it did not show a significant inhibitory effect on *A. tumefaciens* in the 40~60 cm soil layer. As can be seen in Table 6, the control effect of zhongshengmycin was higher than that of Oligosaccharide–Ethylicin, especially at 40–60 cm.

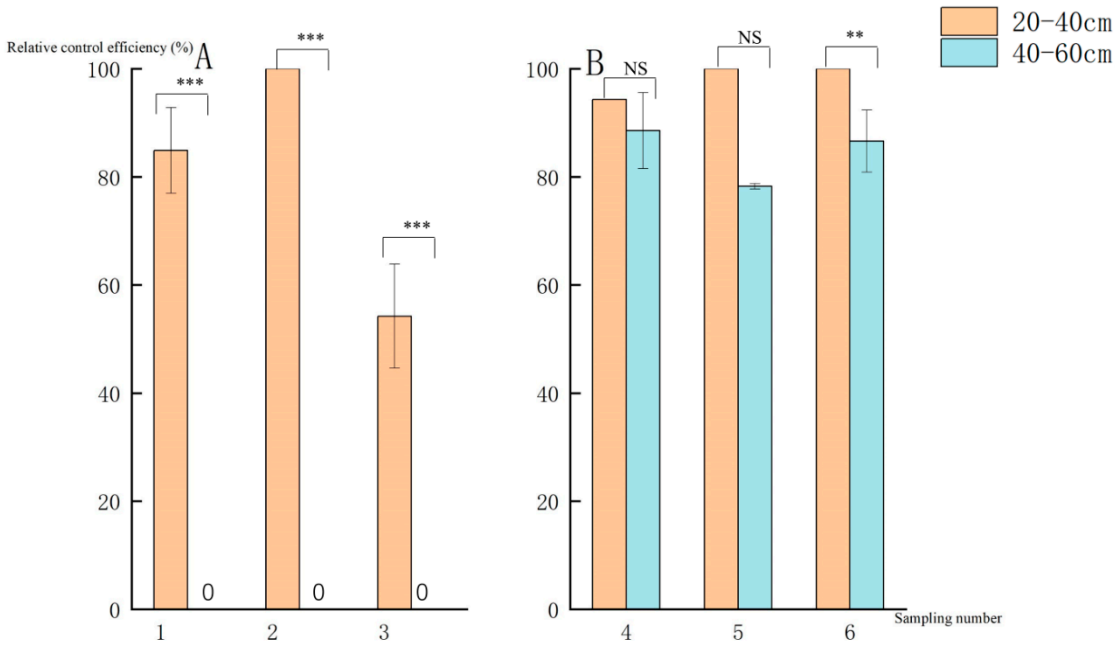

Figure 7. The relative control effect of the two agents. (**A**) represents the control effect of Oligosaccharide–Ethylicin, and (**B**) represents the control effect of zhongshengmycin. Significance is indicated by asterisks, where ** $p < 0.01$, *** $p < 0.001$. NS means not significant.

Table 6. Average efficacy of the two agents.

Pharmaceutical Name	20~40 (cm)	40~60 (cm)
25% Oligosaccharide–Ethylicin (1000×)	79.71 ± 13.47	0
3% Zhongshengmycin (600×)	98.09 ± 1.91 ^{NS}	84.47 ± 3.16 **

** Represents $p < 0.01$, both represent significance, ^{NS} means the difference is not significant.

4. Discussion

4.1. Detection and Distribution of Soil Bacteria

In this study, conventional PCR and qPCR were used to detect *A. tumefaciens* in the soil of cherry trees in Yuyuantan Park. The bacteria was mainly distributed in the soil layer below 20 cm, and its presence increased between 20~60 cm with depth. This result is in line with the distribution characteristics of *A. tumefaciens* in the soil studied by Ren Xinzheng et al. [22].

Crown gall was observed in Yuyuantan Park as early as 1972 when Japanese cherry blossom varieties were introduced [19]. To combat this disease, the park regularly used bio-control agents to inhibit *A. tumefaciens*. These bacteria multiply and become the dominant flora, thereby altering the surface soil microbial community structure [23,24]. However, *A. tumefaciens* has remained latent in the deeper soil layers. Cherry blossom crown gall disease is a cumulative epidemic [25]. As the pathogen accumulated over the years, crown gall disease began to occur in the park, and the disease severity increases [26] year by year.

4.2. Control Methods for Agrobacterium tumefaciens in Cherry Tree Soil

The spread, proliferation, and infection of crown gall disease are all carried out in the soil. After the bacteria invade through a wound, tumors of varying sizes form on the roots and lateral roots, hindering plant nutrient transportation and eventually weakening the plant until its death [4,27,28]. It takes several weeks or more for the disease to develop visible symptoms, and the tumors first appear underground, making them difficult to detect, posing a severe threat to the fruit industry [28,29]. Currently, the prevention and control of crown gall disease mainly includes the breeding of disease-resistant varieties,

physical resection, chemical control, and biological control [30]. Among these methods, chemical control mainly involves root dipping, drenching, or applying agents directly to the tumors. The most common practice is tumor scraping followed by chemical treatments. This method can effectively inhibit the occurrence of root tumors in the short term, but it can greatly damage the root tissue, affecting the growth of cherry trees [31]. Root dipping and drenching mostly affect the soil surface, but because the root system of cherry trees reaches a deep soil layer, surface drenching cannot achieve ideal prevention of crown gall. In this study, zhongshengmycin and Oligosaccharide–Ethylicin were shown to have poor diffusion in soil and a poor effect on soil bacteria in deep soil. After 50 days of Oligosaccharide–Ethylicin drenching, the control efficacy in the 20~40 cm soil layer was between 50% and 100%, but in soil layers below 40 cm, the bacterial load increased, possibly due to degradation of the agent and the migration of bacterial strains, which requires further study.

Establishing a pathogen-free seedling base is the most effective measure to prevent and control cherry crown gall disease [32]. A study by He et al. [33] showed that a combination of methyl bromide fumigation and root immersion treatment achieved a 100% control rate for crown gall disease. However, methyl bromide is an ozone-depleting substance and can cause serious damage to human health, so its use is highly regulated [34,35]. In this study, the replanted soil was fumigated with dazomet and abamectin, and the control efficacy was over 90%. Dazomet is a solid soil fumigant with broad application in China [36]. When applied to wet soil, it produces the active ingredient methyl isothiocyanate (methyl isothiocyanate, MITC), which has a good prevention effect against soil-borne pathogens and weeds [37]. A study by Fang et al. [38] indicated that dazomet, when applied with conventional methods, mainly penetrates the soil layer above 40 cm. In this experiment, a layered application of dazomet achieved good control of soil bacteria in all soil layers, with control efficacy exceeding 90% except for in the 40–60 cm layer in the Wan Ying Garden. In that garden, the presence of many stones in the soil led to problematic soil aeration, causing the MITC to escape and reducing the control efficacy.

5. Conclusions

In this study, primer B1F8 was shown to be suitable for absolute fluorescence quantification of *Agrobacterium tumefaciens* in the soil of Yuyuantan Park, at the annealing temperature of 52 °C and with 38 cycles. *A. tumefaciens* was mainly distributed in the soil layer below 20 cm, and the amount increased with depth. It was found everywhere within a radius of 150 cm from the trunk.

In this study, zhongshengmycin and Oligosaccharide–Ethylicin were used to treat cherry trees showing weak growth and suspected infection. They had good control effects in the 20~40 cm soil layer. Before planting, the replanting soil was fumigated with dazomet and abamectin. This treatment had good control effects in the 0~20 cm, 20~40 cm, and 40~60 cm soil layers.

Crown gall prevention and control has been a serious challenge in forestry production. Understanding the distribution of *A. tumefaciens* in soil is crucial for developing effective field management strategies. For large forest plants with extensive root systems, *A. tumefaciens* is often found deep and widespread in the soil, making daily garden maintenance methods ineffective. Therefore, there is a need to develop new, efficient prevention and control technologies. By improving the conventional application method, one can let the contact agent penetrate the root of the plant and fully contact the pathogen, effectively killing the pathogen. At the same time, the selection of appropriate agents, according to the occurrence of diseases, is important for prevention and control.

Author Contributions: Conceptualization, S.L., Q.H. and A.C.; methodology, S.L., Q.H. and A.C.; software, S.L., Y.Z. and S.G.; validation, S.L., K.Y. and X.Z.; formal analysis, S.L., S.G., Y.Z., Q.H. and A.C.; investigation, S.L., S.G., K.Y. and X.Z.; resources, A.C., X.Z. and Q.H.; data curation, Y.Z.; writing—original draft preparation, S.L.; writing—review and editing, A.C.; visualization, A.C.; supervision, A.C. and X.Z.; project administration, A.C.; funding acquisition, A.C. and X.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Beijing Science and Technology Innovation Project for High-quality Development of Landscaping (KJCX202338) and The Hainan Key Research and Development Program (ZDYF2022XDNY336).

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are contained within this article.

Acknowledgments: We would like to give special thanks to Zhao-ai Shi and Shi-fang Li for their technical support in this experiment.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Ni, D.; Zhang, B. Identification and control of pathogenic bacteria of sakura crown gall in Japan. *Microbiol. China* **1999**, *26*, 4.
2. Shen, J.; Yang, P.; Wang, Q.; Mao, C. Occurrence and risk assessment of domestic forest plant quarantine objects in Zhejiang Province. *Plant Quar.* **1998**, *12*, 4.
3. Xie, Y.; Peng, Q.; Ji, Y.; Xie, A.; Yang, L.; Mu, S.; Li, Z.; He, T.; Xiao, Y.; Zhao, J.; et al. Isolation and Identification of Antibacterial Bioactive Compounds from *Bacillus megaterium* L2. *Front. Microbiol.* **2021**, *12*, 645484. [CrossRef]
4. Yu, S. Occurrence conditions and prevention methods of crown gallosis. *Hebei Fruit Tree* **2016**, *1*.
5. Ma, D.; Wang, H. Root cancer of fruit trees and its biological control. *Chin. Fruit Tree* **1995**, 42–44.
6. You, J.; Ma, D. Study on prevention and treatment of grape root cancerosis by radiosoil Bacillus. *Chin. Fruit Tree* **1993**, *3*.
7. Zhang, C.; Lu, S.; Qian, G. Screening of antagonistic strains of peach root cancer. *Shanghai J. Agric. Sci.* **1988**, 40–45.
8. Wang, H.; Liang, Y.; Yun, T. Introduction of biocontrol agents to control the root cancer of fruit trees in China I. Basic investigation of the disease and isolation and identification of pathogenic bacteria. *J. Beijing Agric. Univ.* **1991**.
9. Li, S.; Zhang, Y. The occurrence and prevention of root cancerosis of fruit trees. *Southwest Hortic.* **2004**, *32*, 2.
10. Lai, E.-M.; Kado, C. Processes VirB2 is the major subunit of promiscuous pilus of *Agrobacterium tumefaciens*. *J. Bacteriol.* **1998**, *180*, 2711. [CrossRef]
11. Hatoh, K.; Izumitsu, K.; Morita, A.; Shimizu, K.; Ohta, A.; Kawai, M. Transformation of the mushroom species *Hypsizigus marmoreus*, *Flammulina velutipes*, and *Grifola frondosa* by an Agrobacterium-mediated method using a universal transformation plasmid. *Mycoscience* **2013**, *54*, 8–12. [CrossRef]
12. Lee, H.; Humann, J.L.; Pitrak, J.S.; Cuperus, J.T.; Parks, T.D.; Whistler, C.A. translation start sequences affect the efficiency of silencing of *Agrobacterium tumefaciens* t- dna oncogenes. *Plant Physiol.* **2019**, *133*, 966–977. [CrossRef]
13. Yakabe, L.E.; Parker, S.R.; Kluepfel, D.A. Role of Systemic *Agrobacterium tumefaciens* Populations in Crown Gall Incidence on the Walnut Hybrid Rootstock Paradox. *Plant Dis.* **2012**, *96*, 1415–1421. [CrossRef]
14. Feng, Z. *Identification of Pathogen of Apple Crown Gall and Establishment of Rapid Detection System of Soil Bacterium for Crown Gall*; China Agricultural University: Beijing, China, 2022.
15. Tan, B.S.; Yabuki, J.; Matsumoto, S.; Kageyama, K.; Fukui, H. PCR primers for identification of opine types of *Agrobacterium tumefaciens* in Japan. *J. Gen. Plant Pathol.* **2003**, *69*, 258–266. [CrossRef]
16. Haas, J.H.; Moore, L.W.; Ream, W.; Manulis, S. Universal PCR primers for detection of phytopathogenic Agrobacterium strains. *Appl. Environ. Microbiol.* **1995**, *61*, 2879–2884. [CrossRef]
17. Weisburg, W.G.; Barns, S.M.; Pelletier, D.A.; Lane, D.J. 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol.* **1991**, *173*, 697–703. [CrossRef]
18. Shi, J. *Molecular Detection Technology of Kiwifruit Crown Gall Bacteria*; Guizhou University: Guiyang, China, 2022.
19. Barker, R.F.; Idler, K.B.; Thompson, D.V.; Kemp, J.D. Nucleotide sequence of the T-DNA region from the *Agrobacterium tumefaciens* octopine Ti plasmid pTi15955. *Plant Mol. Biol.* **1983**, *2*, 335–350. [CrossRef]
20. Li, Q.; Guo, R.J.; Li, S.D.; Li, S.F.; Wang, H.Q. Determination of tumorigenic Agrobacterium density in soil by real-time PCR assay and its effect on crown gall disease severity. *Eur. J. Plant Pathol.* **2015**, *142*, 25–36. [CrossRef]
21. Yang, S.; Sun, Y.; Zhao, X.; Wang, Q.; Bai, Q.; Fang, W.; Cao, A. Soil Quality Investigation and Improvement Measures in Cerasus Plantations in Beijing Yuyuantan Park. *Zhejiang For. Sci. Technol.* **2023**, *43*, 38–44.
22. Ren, X.; Luo, C.; Yang, G. Identification of new biological varieties of *Agrobacterium tumefaciens*. *J. Plant Pathol.* **1990**, 37–42.
23. Ainley, W.M.; Mcneil, K.J.; Hill, J.W.; Lingle, W.L.; Simpson, R.B.; Brenner, M.L.; Nagao, R.T.; Key, J.L. Regulatable endogenous production of cytokinins up to toxic levels in transgenic plants and plant tissues. *Plant Mol. Biol.* **1993**, *22*, 13–23. [CrossRef]
24. Wang, H.; Li, J.; Sui, X.; Wang, J. Rose crown gall pathogens and their sensitive bio-control bacteria. *J. China Agric. Univ.* **1998**, *5*.
25. Zhang, J.; Zhou, J.; Xiang, W. Study on the types of Agrobacterium and its sensitivity to agrobacillin in Populus populus root carcinoma in China. *J. Microbiol.* **1988**, 16–22+103.
26. Wang, Z.; Jin, Y.; Tian, Z.; Wang, G.; Lin, L. Identification of the pathogen from Cerasus crown gall in Ningbo. *J. Plant Prot.* **2014**, *40*, 147–150+164.
27. Zhao, X.; Hou, S.; Sun, Y.; Zhang, G.; Gao, J. Investigation on the Incidence of Cerasus Tree Root Nodule Disease and Evaluation of Dfferent Treatments on Cerasus Tree Root Nodule Disease in Yuyuantan Park. *J. Anhui Agric. Sci.* **2022**, *50*, 138–139+142.

28. Wang, Y. First Report of Crown Gall, Caused by *Agrobacterium tumefaciens* on Soapberry in China. *Plant Dis.* **2013**, *97*, 685. [CrossRef]
29. Carstens, E.; Dawood, Z.; Mansvelt, E.L.; Serfontein, S.; Malan, D.G. First Report of Crown Gall, Caused by *Agrobacterium tumefaciens* on Geraldton Wax (*Chamelaucium uncinatum*) in South Africa. *Plant Dis.* **1999**, *83*, 783. [CrossRef]
30. Yang, W.; Zhang, Z.; Li, S. In henan province and gansu province pear tree crown gall pathogen identification. *J. Plant Prot.* **2020**, *46*, 55–59.
31. Li, S.; Zhang, P.; Li, J.; Sun, Q.; Zhang, F. Analysis of cherry three major diseases—Crown gall, root neck rot, flow gum. *Fruit Trees Yantai* **2017**, 27–30.
32. Yuan, Z.; Zhang, Q.; Zhao, J.; Jiang, X.; Li, Y.; Zhang, X. Progress in cherry crown gall disease. *Fruit Trees China* **2024**, 41–46+57. [CrossRef]
33. He, Y.; Chi, S.; Zhao, B.; Li, B.; Wang, Y.; Wang, Z. Evaluation of Effect of Methyl Bromide Soil Fumigation & Pesticide Root Soaking in Control of *Agrobacterium tumefaciens*. *Zhejiang For. Sci. Technol.* **2011**, *31*, 60–62.
34. Yua, P.; Wang, G.; Chen, Z.; Jin, W. The preliminary research on the degassing velocity of container fumigation with methyl bromide. *Phytoquarantine* **2006**, 209–212. [CrossRef]
35. Dang, A.; Pei, Z. Study on the Dynamics of Environmental Concentrations of Methyl Bromide and Other Fumigants. *Port Health Control* **2024**, *29*, 10–13.
36. Yang, S.; Fang, W.; Bai, Q.; Wang, Q.; Yan, D.; Cao, A. Effect of dazomet fumigation on nitrogen uptake and utilization in tobacco. *J. Plant Prot.* **2024**, *50*, 100–110.
37. Fang, W.; Wang, Q.; Yan, D.; Cao, B.; Xu, J.; Jin, X. Research progresses and future development trends of soil fumigant dazomet in control of soil-borne diseases. *J. Plant Prot.* **2023**, *50*, 40–49.
38. Fang, W.; Cao, A.; Wang, Q.; Li, Y.; Jin, X.; Qiu, Y.; Zhao, Q.; Zhao, H. A New Integrated Soil Disinfection Machine Improves the Uniformity of Dazomet in Soil. *Sci. Agric. Sin.* **2021**, *54*, 2570–2580.