

Article

Field Efficacy of Fluopyram Suspension Concentrate against Pine Wilt Disease and Its Distribution and Persistence in Pine Tree Tissues

Yajie Guo ^{1,2,3,†}, Jiayi Ma ^{1,2,4,†}, Ziyi You ^{1,2,†}, Mingqing Weng ^{1,2}, Rebeca Carballar-Lejarazú ⁵, Wenlong Jiao ^{1,2}, Jieqin Wu ¹, Xia Hu ^{1,2}, Rong Wang ^{1,2} , Feiping Zhang ^{1,2} and Songqing Wu ^{1,2,6,*} 

- ¹ Forestry College, Fujian Agriculture and Forestry University, Fuzhou 350002, China
² Key Laboratory of Integrated Pest Management in Ecological Forests, Fujian Agriculture and Forestry University, Fuzhou 350002, China
³ Laboratory of Forest Symbiology, Asian Research Center for Bioresource and Environmental Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo 188-0002, Japan
⁴ Pharmaceutical and Medical Technology College, Putian University, Fuzhou 350002, China
⁵ Department of Microbiology & Molecular Genetics, University of California Irvine, Irvine, CA 92697-4025, USA
⁶ State Key Laboratory of Ecological Pest Control for Fujian and Taiwan Crops, College of Forestry, Fujian Agriculture and Forestry University, Putian 351100, China
* Correspondence: dabinyang@126.com
† These authors contributed equally to this work.

Abstract: Fluopyram is a novel nematicide compound with systemic activity, and it is a potential chemical agent for controlling pine wilt disease, which is devastating pine trees worldwide. Here, a fluopyram suspension concentrate (SC) was used to determine its efficacy in controlling pine wilt disease, its distribution in pine tree tissues, and its environmental degradation. Fluopyram SC had an evident inhibitory effect on *Pestalotiopsis* sp. and showed a good preventive effect on pine wilt disease in healthy pine seedlings. In the field application, after spraying onto the canopy of pine trees, fluopyram was absorbed and translocated downward to all parts of the tree via the branches and needles, and its distribution was stable at 30 days post-application (all samples' mean residual concentration = 1.166 mg/kg). In addition, the maximum concentration of fluopyram residue in soil and water was 1.139 and 0.099 mg/kg, respectively, at 30 days post-application when fluopyram was significantly degraded. The control efficiency of fluopyram SC in the forest reached 90.48% when the formulation was sprayed by agricultural unmanned aerial vehicles. The half-life of fluopyram SC was 346.6 days, with a persistence of 3 years. These results indicate that spraying fluopyram SC could effectively reduce the number of pine trees dying from pine wilt disease.

Keywords: pine wilt disease; fluopyram; suspension concentrate; field control effect; distribution; persistence



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1. Introduction

Pine wilt disease (PWD) is caused by the pinewood nematode (PWN), *Bursaphelenchus xylophilus* (Steiner et Buhner) Nickle, and results in a high rate of pine tree mortality [1]. This nematode is listed as a quarantinable pest in 52 countries, with Asia being the region with the most serious PWD epidemics. Pine forests in many countries have been severely damaged due to PWN infection and outbreaks, impairing the services and function of these ecosystems and posing a severe general threat to pine forests worldwide [2,3]. Despite various methods to control PWD, including forest management practices, physical, biological, and chemical control, in practice, the most effective control method is to cut and burn the infected and dead pine trees in the forest, but this requires a considerable amount of labor and material resources, and its cost is extremely high [4]. Therefore, current

research in forest pest management is focusing on efficient and cost-effective methods for PWD control.

Currently, chemical agents mainly target PWNs and their insect vectors to control PWD [5]. However, with changing trends in society and the economy, chemical control agents are now expected to have low toxicity, high efficiency, and be environmentally friendly. The traditional, highly toxic organophosphorus nematicides generate unacceptable high levels of residues that can severely harm the environment [6]. Although some chemical pesticides have been applied effectively via the trunk-injection method in forests, this method lacks systemic activity, coupled with the high cost, making it impractical for application in the forest setting [7,8]. Therefore, finding a new high-efficiency and environment-friendly chemical agent capable of having a systemic activity to resolve the problems associated with PWD control is imperative.

Fluopyram is a succinate dehydrogenase inhibitor (SDHI) in the phenyl-benzamide chemical group, which is known to move systemically through foliage acropetally [9,10]. Fluopyram was originally developed as a fungicide but could also be used as a nematicide [11,12]. Importantly, fluopyram exhibits significant nematicidal activity against *B. xylophilus*; therefore, given its robust systemic activity in trees, fluopyram could be a promising candidate trunk-injection agent for controlling PWN [8]. Nevertheless, reliance on the trunk injection method is still expensive. Considering the systemic activity of fluopyram, the key question becomes, does fluopyram move throughout the tree once absorbed by branches and leaves after aerial spraying it in the pine forest?

To determine whether fluopyram can be absorbed and translocated throughout the tree after spraying, we used a fluopyram suspension concentrate (SC) to control PWD effectively. In this study, we quantified the translocation within trees and duration of the nematicidal effect of fluopyram in *Pinus massoniana* trees and further evaluated the control effects of fluopyram SC by detecting its residual concentration in branches, needles, soil, and water after spraying it. The diffusibility and persistence of fluopyram SC in the environment were also analyzed as well as the dynamic digestion of fluopyram in *P. massoniana*. Using the results, we assessed the efficiency of fluopyram SC in controlling PWD in pine forests.

2. Materials and Methods

2.1. Field Study Sites and Nematicide Application

The efficiency of fluopyram SC in the field was conducted in 2019 at four sample plots, including Qingkou, Minhou County, China (25°52'9.00" N, 119°22'21.03" E) (sample plot 1), Jin'an District, Fuzhou City, China (26°6'42.46" N, 119°23'24.47" E) (sample plot 2), Hongwei, Minhou County, China (26°10'58.60" N, 119°1'14.04" E) (sample plot 3), Wuyishan National Park, China (27°38'11.54" N, 117°55'58.62" E) (sample plot 4). The tests of the distribution of fluopyram in trees and the degradation of fluopyram in soil and water samples were performed at Hongwei, Minhou, Fuzhou, Fujian (26°9'11.40" N, 118°57'32.06" E) (sample plot 5). *P. massoniana* forest in all sample plots are mixed stands of natural forest and planted forest, the stand age is about 40 years old, and the canopy density of all sample plots is >0.7. These sampling plots are all located in endemic areas of PWD, which are announced by the government [13]. The city or county for sample plots 3 and 4 was a PWD endemic area in 2021 only, while sample plots 1 and 2 are located in areas that have been affected by PWD for the past 5 years. Healthy *P. massoniana* with 20–30 cm diameter at breast height (DBH) was used for the test in all sample plots (additional information in Table S1).

The formulation of fluopyram SC used in this study was Lufuda® (41.7% fluopyram SC, Bayer CropScience China Co., Ltd., Fuzhou, China). The compound was diluted with water into a solution for spraying, containing 1% fluopyram active ingredient. For its forest application, the T20 plant protection UAV (unmanned aerial vehicle) (Shenzhen DJI Sciences and Technologies Ltd., Shenzhen, China) was used to spray the fluopyram SC on the pine canopy at 1 kg/ha.

2.2. Experiment 1: Fungistatic Effect of Fluopyram

PWN can survive in nature by feeding on *Pestalotiopsis* sp. and *Ceratocystis* sp. [14,15]. These two fungi are also the dominant fungi isolated from dead pine wood infected by PWD. The growth rate method was used to determine the fungistatic effect of fluopyram on *Pestalotiopsis* sp. and *Ceratocystis* sp. Briefly, fluopyram was diluted with double-distilled water into five different concentrations: 0.041700, 0.010425, 0.002606, 0.000652, and 0.000163 mg/L. Then, 2 mL of the suspension was added to a 20 mL Potato-dextrose agar (PDA) medium. PDA medium with 2 mL of double-distilled water was used as a control. A 0.6 cm diameter fungus cake was added into the center of each PDA plate and then cultured in an incubator at 28 °C. The growth of the fungi in different concentrations was recorded. All concentrations were tested in triplicate. After the mycelium of the control group had grown over the entire plate, the diameter of the mycelia was measured in all treatments by the diameter measured on two perpendicular axes, and the inhibition rate of mycelia growth was calculated as follows:

$$\text{Diameter of colony growth} = (\text{horizontal diameter} + \text{longitudinal diameter})/2 - 0.6 \quad (1a)$$

$$\text{Inhibition rate of mycelia growth (\%)} = (\text{Diameter of the colony in the control group} - \text{Diameter of the colony in the treatment group})/\text{Diameter of the colony in the control group} \times 100 \quad (1b)$$

2.3. Experiment 2: Suppression of Pine Wilt Disease in the Laboratory

The control efficacy of fluopyram SC to PWD was evaluated on pot-grown 3-year-old healthy pine seedlings. The seedlings were planted in pots 18 cm in diameter with soil and placed in a shade house to grow. Then, 41.7% fluopyram SC was diluted 15,000 folds with distilled water before use, and 450 mL of the 15,000-fold dilution was sprayed on each seedling, and 10 seedlings were treated. Ten seedlings were sprayed with water as control. One week after fluopyram SC or water application, PWNs were inoculated by inoculation under the bark [16]. Briefly, in the central part of the seedling, about 10 cm above the base, a small slit about 0.5 cm in length was cut at an oblique angle of 30° with a scalpel cutting the xylem. Then, a cotton ball was placed in the wound, sealed with parafilm, and slowly injected with PWN suspension through a pipette. Each seedling was inoculated with approximately 3000 nematodes. The incidence of pine seedling disease was observed and recorded until all control seedlings had died (about 60 days after inoculation).

To determine whether PWD caused the death of the inoculated seedlings, the branches of all seedlings were collected, and the PWN in branches was detected by the Baermann funnel method [17,18]. Briefly, the Baermann funnel apparatus consisted of a funnel with an inner diameter of 12 cm and a 10 cm piece of silicone hose, which was tightly attached to the funnel end. The end of the silicone hose was closed with a squeezer clip. Three paper towels were in the funnel to prevent samples from entering the nematode solution. Branches samples were cut into small chips and put on the paper; then, the distilled water was added into the funnel just above the paper towel. This apparatus was installed in a horizontal position for 24 h at room temperature. The nematodes were separated from the samples and accumulated at the end of the silicone hose. Then, nematodes were collected into a 10 mL tube and verified using a compound microscope.

2.4. Experiment 3: Distribution of Fluopyram Suspension Concentrate in Trees

At sample plot 5, 1% fluopyram SC was sprayed by the T20 plant protection UAV on the pine canopy at 1 kg/ha in May 2019, and six pine trees in this plot were randomly selected to measure the residual concentration of fluopyram. The fluopyram residue tests were carried out on these trees at 7 and 30 days post-application.

Briefly, three trees were selected for destructive sampling at 7 days post-application, and the remaining three were destructively sampled at 30 days post-application, as shown in Figure 1. The branches and needles at the approximate mid-point between the base of the crown and the top of the tree were collected and detected for fluopyram residual

concentration, respectively. The trunk samples were collected from approximately 1 to 1.5 m above the ground surface, and their sapwood and duramen were detected separately. For each sample position, four samples, one from each cardinal direction, were collected.

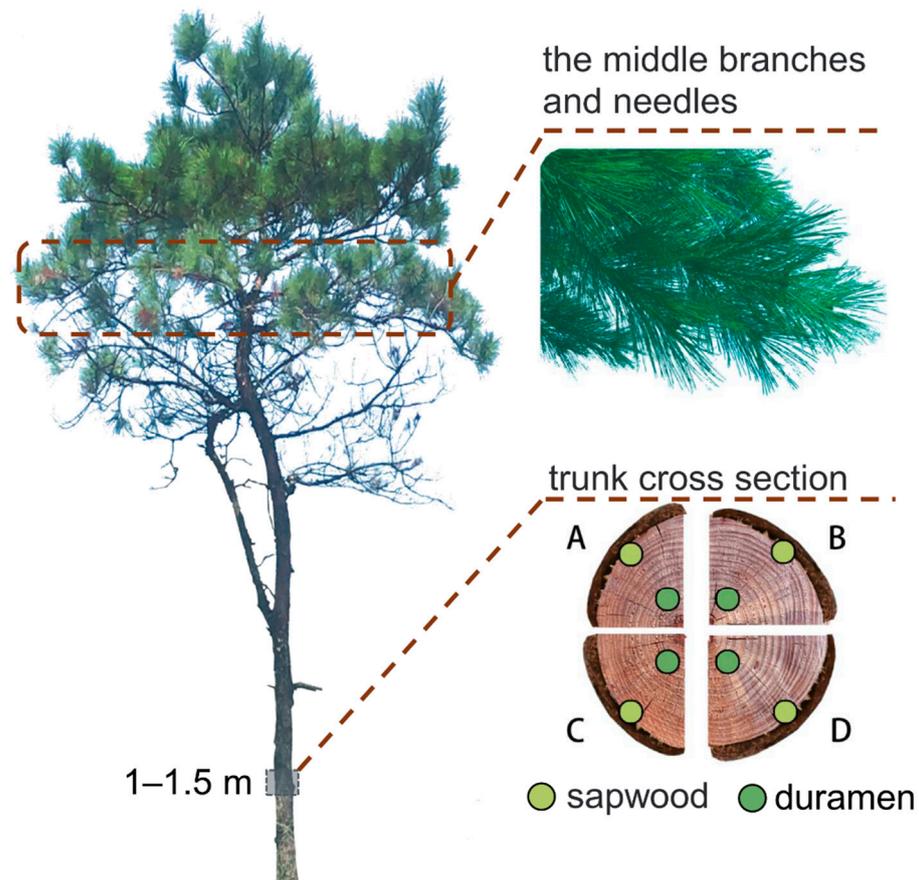


Figure 1. Sampling diagram from fluopyram suspension concentrate treated *Pinus massoniana*. A–D represent the four cardinal directions of sampling.

2.5. Experiment 4: Movement and Persistence of Fluopyram in Soil and Water

To test the fluopyram residual concentration in the environmental samples of pine forest, 1% fluopyram SC was applied in May 2021 in the PWD epidemic area of sample plot 5. Soil and water samples were collected at 7 and 30 days post-application of fluopyram SC. Three routes (A–C) were selected around the sample plot, and four sampling points with different distances were set on each route, including from the sample plot (i.e., 0 m) and 10 m, 50 m, and 200 m from its edge. There is no water at the 0 m site, so only soil samples are collected here. Three samples were randomly collected at each sampling point. Water samples were collected from springs in the pine forest and reservoirs outside the treated areas (Figure S1). The sampling sites are located in a mountain area with an altitude of about 90–120 m (26°8′59.20″ N, 118°57′14.27″ E), and the soil nature is krasnozem. The annual average temperature is about 18–20 °C, and the annual average relative humidity is 75%–80%. Briefly, about 5 g soil samples from 0–5 cm below the surface were collected using collection ziplock bags, and 50 mL water samples from the stream in the plots were collected in 50 mL plastic tubes. The samples from the untreated plots were used as controls. All samples were placed in dry ice immediately after collection, brought back to the laboratory, and stored at –80 °C until use.

2.6. Experiment 5: Efficacy of Aerial-Applied Fluopyram in Controlling PWN Infestations

To evaluate the control efficacy of fluopyram against PWD in the field, fluopyram SC was sprayed in four sampling plots from 2019 to 2021. The crowns of *P. massoniana*

trees were sprayed with fluopyram SC (1% active ingredient) at an application rate of 1 kg/ha by the T20 plant protection UAV in the morning on a sunny day. The blocks in four sample plots without SC spray were used as controls. The control efficacy was recorded and calculated over three successive years, 2019–2021 (Figure 2, more detailed information in Table S1).

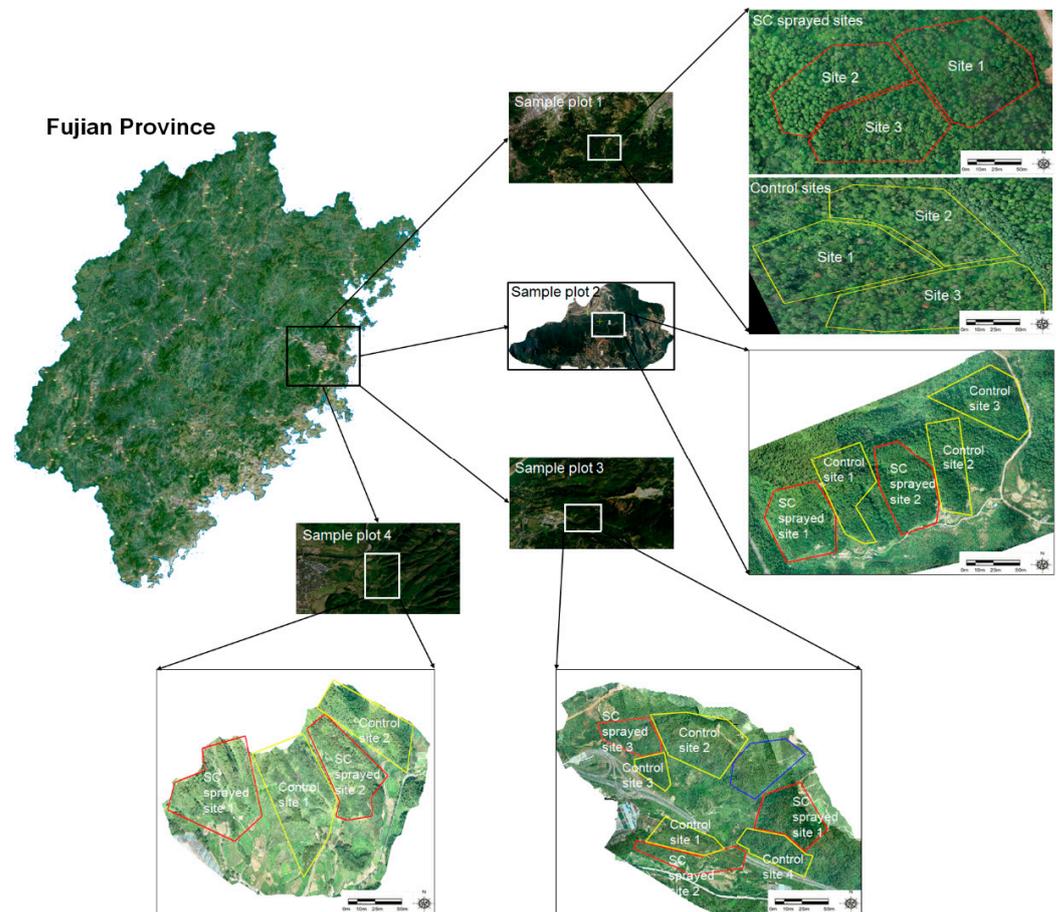


Figure 2. Map of sampling plots used for spraying fluopyram suspension concentrate. Plot 1: Qingkou, Minhou County, China ($25^{\circ}52'9.00''$ N, $119^{\circ}22'21.03''$ E). Plot 2: Jin'an District, Fuzhou City, China ($26^{\circ}6'42.46''$ N, $119^{\circ}23'24.47''$ E). Plot 3: Hongwei, Minhou County, China ($26^{\circ}10'58.60''$ N, $119^{\circ}1'14.04''$ E). Plot 4: Wuyishan National Park, China ($27^{\circ}38'11.54''$ N, $117^{\circ}55'58.62''$ E).

In detail, sample plot 1 was sprayed in May 2019, 6 blocks with an area of 0.67 ha were selected, and three blocks each were in the control and sprayed groups. To detect the fluopyram residue in trees, the four main directions' branches with needles, at the approximate mid-point between the base of the crown and the top of the tree (middle branches), were collected from three to four randomly selected trees at 90, 455, and 820 d after SC application. Likewise, two sprayed blocks and three control blocks in sample plot 2 were sprayed in June 2020; the area of each block was 1.67 ha. The branches' sampling times were 90 and 455 d after the application. Sample plots 3 and 4 were sprayed in May and July 2021, respectively. In sample plot 3, three blocks with an area of about 3.33 to 13.33 ha were selected for spraying, and four blocks were used as control groups. While sample plot 4 includes two sprayed blocks and two control blocks (about 5.33 to 16.67 ha each). Branch samples from sample plots 3 and 4 were collected at 90 d after SC application.

Before spraying, the initial number of dead woods was counted in each sample plot. After the spray of fluopyram SC, the death of *P. massoniana* individuals was registered and counted in the sprayed and control blocks in November of every year. A P4 Multispectral unit (Shenzhen DJI Sciences and Technologies Ltd., Shenzhen, China) was used to take

the photographs positioned at 150–200 m of height in the sprayed and control plots, then suspected dead trees were marked according to the regional map that was spliced by the photographs. According to the recognized regional distribution pictures of dead pine trees, a field confirmation was conducted in person in the sample plot and all dead pine trees were counted. The 1 or 2 middle branches of all dead pine trees were collected, and the PWN in branches was determined by the Baermann funnel method in the laboratory to corroborate that those pine trees were killed by PWD. The efficiency of fluopyram SC to control PWD was calculated by using the following formula [19]:

$$\text{Increase in dead pine trees (\%)} = (\text{Number of new dead woods} / \text{Total number of test pine trees}) \times 100 \quad (2a)$$

$$\begin{aligned} & \text{Control efficiency of PWD (\%)} = (\text{Increase in dead pine trees in the control group} \\ & - \text{Increase in dead pine trees in the treatment group}) / \text{Increase in dead pine trees in the control group} \times 100 \end{aligned} \quad (2b)$$

2.7. Experiment 6: Degradation Kinetics Analysis of Fluopyram in Trees

Combined with the data of the distribution test of fluopyram SC in the trees and the field experiments in sample plot 1, the fluopyram residue in the middle branch samples was determined at 0, 7, 30, 90, 455, and 820 d after fluopyram SC application. Excel 2020 software was used to analyze the fluopyram residual concentration data, and the degradation kinetics equation and degradation half-life of fluopyram in *P. massoniana* were calculated as follows [20]:

$$C_t = C_0 e^{-kt} \quad (3a)$$

$$\text{Half-life } (t_{1/2}) = \frac{\ln 2}{k} \quad (3b)$$

where C_0 is the residual concentration of fluopyram at 0 days (mg/kg); C_t is the residual concentration of fluopyram at day t (mg/kg); and k is the degradation kinetic constant.

2.8. Sample Preparation for GC-MS

Samples were pre-processed before GC-MS detection. The branches, needles, and trunk samples from trees were chopped into more minor chips. Each tissue sample was homogenized with methanol (100 mL) for 10 min (in a homogenizer (Dongguan Fangtai Electric Appliance Co., Ltd., Dongguan, China)). Soil samples were ground into powder, and the same analytical procedures were used for both the plant tissue samples and the soil samples. In detail, 1 g of the homogenized mixture or soil as the powder was added into a 50 mL tube with 10 mL acetonitrile, 2 g of MgSO_4 , 1 g of NaCl, and 20 mg of activated carbon. After shaking and mixing with a digital Vortex4 (Shenzhen Sanli Chemical Co., Shenzhen, China) for 1 min, the homogenized mixture was sonicated using an Ultrasonic Bath (KH-300DE, Kunshan Ultrasonic Instrument Co., Ltd., Kunshan, Jiangsu, China) for 10 min and centrifuged at $5000 \times g$ for 10 min. The supernatant (6 mL in total) was mixed with 400 mg of MgSO_4 and 150 mg of N-propyl ethylenediamine (PSA, Welch Materials, Ellocott City, MD, USA), sonicated for 10 min, and centrifuged at $5000 \times g$ for 10 min after shaking and mixing for 1 min. Two mL of the resultant supernatant was evaporated to dryness under a nitrogen purge (N-EVAP-45, Organomation, Berlin, MA, USA) at 60 °C, ensuring that no moisture entered the tube. Finally, each sample was resuspended to a 1 mL volume with hexane and filtered through a 0.22 μm organic membrane for its GC-MS analysis.

From each water sample, 10 mL were filtered to remove any solid impurities, and the water was subjected to vortex oscillation for 10 min with 10 mL of n-hexane. This mixture was sonicated for 10 min and centrifuged at $5000 \times g$ for 5 min. The supernatant of the n-hexane solution was collected into a 10 mL tube, dried under a nitrogen purge, and finally resuspended with methanol to a volume of 1 mL. Each sample was filtered through a 0.22 μm organic membrane for its GC-MS analysis.

2.9. GC-MS Instrumentation and Chromatographic Conditions

The residual concentration of fluopyram in pine trees, soil, and water samples at 7 and 30 days post-application was statistically analyzed by GC-MS. We used the QP2010 plus gas chromatograph-mass spectrometer (GC-MS, Shimadzu, Shanghai, China) equipped with a GCMS-QP2010 Plus wide-bore sampler (Shimadzu, Shanghai, China). The column used was a DB-5 ms capillary column (30 m × 0.25 mm × 0.25 μm), and the temperature procedure consisted of 80 °C for 1 min + 30 °C/min to 220 °C + 5 °C/min to 240 °C + 10 °C/min to 280 °C, held at 1 min. The mobile phase was nitrogen (purity ≥ 99.999) at a flow rate of 1.0 mL/min; the sample volume was 20 μL at a flow rate of 0.3 mL/min. The electron bombardment (EI) source was 70 eV, the ion source temperature was maintained at 230 °C, and the interface temperature was 290 °C. The solvent delay was 5 min. The SCAN range was 45–550 *m/z*. The flow rate of atomized gas was 1.5 L/min, and the drying pressure was 121 kPa. The residual concentration of fluopyram in each sample was calculated with the following formula:

$$C = \frac{5C_0 \cdot \rho \cdot V}{m} \quad (4)$$

where *C* is the concentration of the pesticide in the sample (mg/kg); *C*₀ is the measured value of the sample (mg/kg); *ρ* is the density of n-hexane (kg/L); *V* is the volume of n-hexane (L); and *m* is the mass of the sample (kg).

2.10. Statistical Analyses

All data were collated and calculated using Microsoft Excel 2020, and all statistics graphs were generated using GraphPad Prism 6 (9.5.0 for Windows). SPSS Statistics (19.0 for Windows) software was used for data analysis. Data from each experiment were tested for homogeneity of variance and one-way analysis of variance (ANOVA, α = 0.05) for comparing the means of the single continuous variable between different groups. Then, data from the fluopyram residue in the tree, soil, and water samples, as well as the inhibition rate of fungal growth, were analyzed by multiple comparisons of the means by Tukey's test (*p* = 0.05) and Duncan's multiple range test were used to make multiple comparisons of the means for the data of forest control efficiency. Values are presented as means ± SE. For the experiment of the fungistatic effect of fluopyram, regression analysis of virulence was performed using concentration and inhibition rate as independent and dependent variables, respectively, and the median effective concentration (EC₅₀) in the maximum effect of inhibition was calculated.

3. Results

3.1. Fungistatic Effect of Fluopyram

The growth of *Pestalotiopsis* sp. and *Ceratocystis* sp. was inhibited by fluopyram SC at different concentrations, and the inhibition effect was more evident in *Pestalotiopsis* sp. (Figure 3). At the same time, the higher the concentration of fluopyram, the higher the inhibition rate of fungal growth (Figure 3). The EC₅₀ of fluopyram SC against *Pestalotiopsis* sp. and *Ceratocystis* sp. were 63.807 mg/L and 496.618 mg/L, respectively.

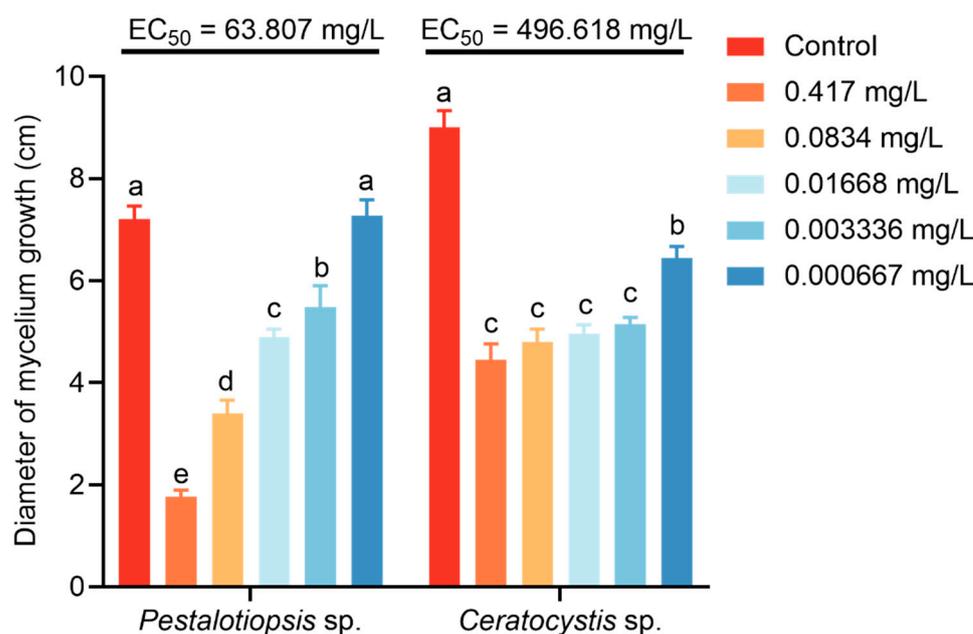


Figure 3. Inhibitory effect of different concentrations of fluopyram on *Pestalotiopsis* sp. and *Ceratocystis* sp. The fungus was cultured by PDA medium without fluopyram as control, and other treatments were cultured on PDA medium with 0.000667 mg/L, 0.003336 mg/L, 0.016680 mg/L, 0.083400 mg/L, and 0.417000 mg/L fluopyram. Bars with different letters indicate statistical significance at $p < 0.05$.

3.2. Suppression of Pine Wilt Disease in the Laboratory

The preventive effect of fluopyram SC on PWD was tested in the laboratory; ten healthy pine seedlings were selected and sprayed with fluopyram SC and then inoculated with 3000 nematodes in the sprayed pine seedlings after 1 week of fluopyram SC application to observe whether the pine seedlings were killed. After 60 days post-infection, all pine seedlings in the fluopyram treatment group were not infected by PWD, and all seedlings in the control group died (Figure 4). A large number of nematodes were detected in the branches of all pine seedlings in the control group. While there were little or no nematodes detected in the fluopyram-sprayed seedlings. The needles of pine seedlings in the control group were russet or reddish brown, which is a symptom of PWD (Figure 4B). Fluopyram SC showed a good preventive effect on PWD in healthy pine trees.

3.3. Distribution of Fluopyram Suspension Concentrate in Trees

The overall analysis of the four tissue parts of the pine trees showed that the fluopyram residual concentration in the branches and needles exceeded the amount in the trunk at both 7 and 30 days post-application. All samples' mean residual concentrations of fluopyram in pine trees at 7 and 30 days post-application were 1.621 ± 1.59 and 1.166 ± 1.09 mg/kg, respectively (Figure 5). Compared with 7 days post-application, the residual concentration in branches, needles, and xylem was significantly reduced at 30 days but still at lower concentrations in the trunks (Figure 5). There was no significant difference in the fluopyram residual concentration in the four cardinal directions. The residual distribution trend of the samples for each cardinal direction was thus consistent with the overall distribution.

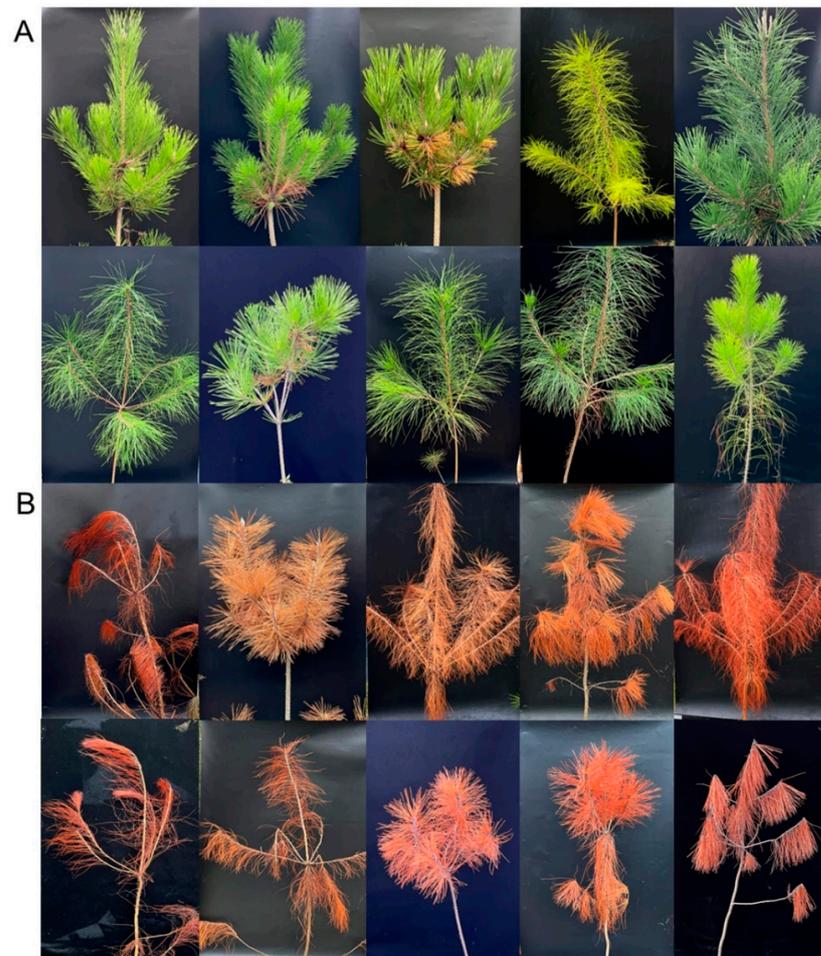


Figure 4. Effect of fluopyram SC application on pine wilt disease in the laboratory. These seedlings were treated with fluopyram DP (A) and water (B), control) for the prevention effect test in the laboratory, and the photos were taken at the end of the observation.

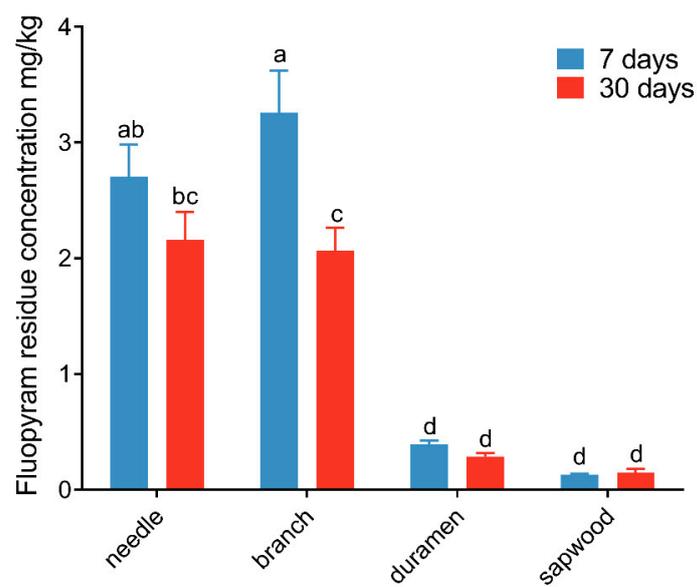


Figure 5. Residual concentration of fluopyram in different samples of *Pinus massoniana* at 7 days and 30 days post-application of the fluopyram suspension concentrate. Bars with different letters indicate statistical significance at $p < 0.05$.

3.4. Movement and Persistence of Fluopyram in Soil and Water

Fluopyram was detected in soil samples from 0 to 50 m away from the sampling plot five at 7 and 30 days post-application. No pesticide residue was detected in soil samples from 200 m away. As the time since application increased, the concentration of fluopyram residue in the soil decreased (Table 1). Furthermore, the residual concentration of fluopyram in water samples from 10 m away in sampling plot 5 (12.056 mg/kg) was significantly higher than that in other samples at 7 days post-application. The water sampling showed that fluopyram did enter the stream and was transported at least 200 m downstream. After 30 days post-application, no pesticide residue was detectable in water samples at other distances, except for a minute amount of residue found in samples taken 10 m away from the plot (Table 1).

Table 1. Fluopyram residue in soil and water samples after applying the suspension concentrate in the forest.

Sample	Distance from the Application Plot (m)	Residual Concentration (mg/kg) (\pm SE)	
		7 Days	30 Days
Soil	0	1.601 \pm 1.97 ^b	1.139 \pm 0.95 ^b
	10	1.412 \pm 1.17 ^b	0.105 \pm 0.05 ^b
	50	0.033 \pm 0.05 ^b	0.011 \pm 0.06 ^b
	200	0 \pm 0 ^b	0 \pm 0 ^b
	Control	0 \pm 0 ^b	0 \pm 0 ^b
Water	10	12.056 \pm 20.20 ^a	0.099 \pm 0.12 ^b
	50	0.121 \pm 0.05 ^b	0 \pm 0 ^b
	200	0.077 \pm 0.07 ^b	0 \pm 0 ^b
	Control	0 \pm 0 ^b	0 \pm 0 ^b

Note: Different letters indicate statistical significance at $p < 0.05$.

3.5. Efficiency of Fluopyram Suspension Concentrate in the Field

According to the yearly statistics of dead pine trees, the average control efficiency of PWD in the three years was 78.73%, 86.65%, and 90.48%, respectively, after spraying fluopyram SC in sampling plot 1. The fluopyram residual concentration in the third year was 0.371 mg/mL; this was markedly higher than the LC₅₀ (0.056 mg/L) of fluopyram for PWN. After 2 years of application in sampling plot 2, the average control efficiency in the first 2 years also surpassed 70%. In the second year, the residual concentration of fluopyram was 0.645 mg/mL (Figure 6; Table S2).

However, in sample plot 3, after the same spraying of fluopyram SC in May, the average control efficiency in the first year only reached 59.8%, significantly lower than sample plot 1 or sample plot 2 in the first year (Figure 6). In addition, the fluopyram SC was sprayed in sample plot 4 later than in other sample plots, and the efficiency in the first year was only 40.72%, lower than that of other sample plots (Figure 6). Nonetheless, following the application, all fluopyram residual concentrations in the pine branches and leaves declined yearly, being nearly higher than the LC₅₀ (0.059 mg/L) of fluopyram against pine nematodes, with only a few exceeding the LC₉₀ (1.026 mg/L) (Table S2).

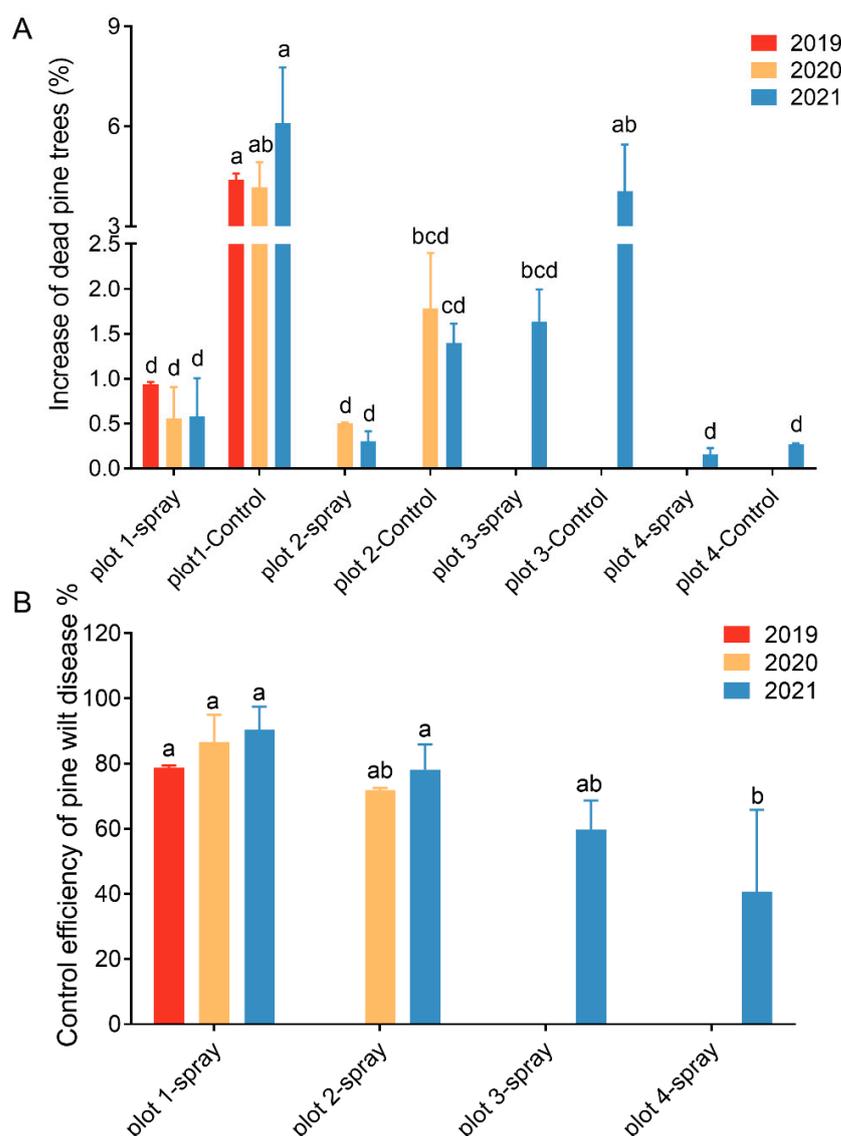


Figure 6. Preventative effect of fluopyram suspension concentrate in the pine forest. Increase in dead pine trees (%) (A) and control efficiency of pine wilt disease (%) (B) in three sample plots in 2019, 2020, and 2021. Bars with different letters indicate statistical significance at $p < 0.05$.

3.6. Degradation Kinetics Analysis of Fluopyram in Trees

Fluopyram residues were detected in pine branches and needles at 0, 7, 30, 90, 455, and 820 d post-application with fluopyram SC. The average fluopyram residual concentrations in branches and needles at 0 d was 0 mg/kg, indicating that 0–7 days post-application the fluopyram was first absorbed and enriched in the top branches and leaves. Coincidentally, the results of fluopyram distribution also indicated that fluopyram was translocated in the tree and distributed in various pine tissues 7 days after application (Figure 5). Thus, the fluopyram degraded by 29.14% from the 7- to 30-day interval, increasing slightly to 34.70% from 30 to 90 days, yet reaching 87.56% at 820 days since the application (Table 2). The kinetic model of fluopyram SC in pine trees was $C_t = 2.524 e^{-0.002t}$ ($r^2 = 0.9338$) by fitting the first-order kinetic equation from 7 days post-application. The results showed that the half-life of the fluopyram SC was 346.6 days, nearly 1 year.

Table 2. The degradation proportion of fluopyram in the branches and needles of pine trees after their treatment with the suspension concentrate.

Days	Residual Concentration (mg/kg)	95% Confidence Interval	Degradation (%)
7	2.98	2.55–3.41	0
30	2.11	1.83–2.40	29.14
90	1.95	1.24–2.66	34.7
455	0.77	0.37–1.16	74.31
820	0.37	0.02–0.73	87.56

4. Discussion

Currently, commercially available chemical pesticides that can be sprayed on trees for PWD control lack systemic activity, and most of them are insecticides used to control the insect vector *Monochamus* spp., so their effect on PWN is limited [21,22]. To overcome these challenges, in this study, fluopyram was used as a suspension concentrate to test its efficiency on PWD control due to its systemic activity. The systemic activity of fluopyram may promote the better, more widespread, and stable transport of this chemical agent in trees [10]. There have been reported other chemicals for nematode control. For example, abamectin is also water-soluble (10 µg/L at 21 °C), but it has a lower water solubility than fluopyram (16 mg/L at 20 °C), which makes its application difficult [10,23]. The efficacy of the avermectin derivative against avocado thrips was poor, and this was likely the result of its low water solubility, which restricted the amount of avermectin that could be absorbed into tree tissues [23]. Emamectin benzoate is another agent used to manage the PWD by trunk injection in Japan, showing a successful distribution and activity against PWN in smaller trees with trunk diameters of less than 15 cm [24]. In the present study, since the suspension concentrate was sprayed onto the canopy of pine trees, the concentrate likely translocated downward to all the tree tissues via the branches and needles. More extended periods post-application showed that fluopyram could be uniformly distributed in each tissue of pine tree with 20–30 cm DBH. Thirty days post-application, the residual concentration of fluopyram in needles and branches was still significantly higher than the LC₅₀ of fluopyram to PWN (0.059 mg/L). Therefore, compared with the existing chemical pesticides used for PWN control, fluopyram SC has a robust systemic activity and transport activity, thus providing a new way to control infestations of PWN effectively.

Fluopyram was initially formulated as a fungicide by Bayer Crop Science (RTP, NC). Its activity against various fungi has been critically assessed, including the ability to control *Fusarium virguliforme* [11]. It is reported that conidia germination of *F. virguliforme* was determined to be more sensitive to fluopyram (mean EC₅₀ = 2.28 µg/mL) than the mycelial growth (mean EC₅₀ = 3.35 µg/mL) [11]. We found that fluopyram has an obvious fungistatic effect on *Pestalotiopsis* sp., on which nematodes feed; the mean EC₅₀ for fluopyram to *Pestalotiopsis* sp. was 63.807 mg/L that lower than the EC₅₀ of *F. virguliforme*. In addition, fluopyram has also been applied as a nematicide against root-knot nematodes, cyst nematodes, and other soil nematodes [12,25–27]. Concerning its effect on nematodes, research has shown that fluopyram provides a good level of control over the root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood, and the reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, for which the 2 h EC₅₀ values are 5.18 and 12.99 µg/mL fluopyram, respectively [25]. Crucially, fluopyram also displays activity against the PWN. For example, fluopyram exhibited significant nematicidal activity, with LC₅₀ values of 0.945, 1.69, and 3.23 mg/L for second-stage juveniles (J₂), third-stage juveniles (J₃), and fourth-stage juveniles/adults (J₄/adults), respectively [8]. In the present study, the results of spraying pine seedlings with fluopyram SC in the laboratory showed that it had an evident preventive effect on PWD, showing the first case that fluopyram spraying can prevent PWD.

It has been suggested that fluopyram might be an alternative agent for use in trunk injections [8]. However, when managing forests with complex terrain across a large area, applying trunk injections is difficult and costly in terms of both the workforce and material

resources required. Therefore, compared with trunk injection, the aerial application of fluopyram SC is more feasible and affordable in forests. In general, SC is a widely used dosage form with many advantages. The solid is non-dusty, disperses well in water with water-soluble adjuvants, is easier to apply, is safe for the operator and environment, and costs relatively less [28]. Applications of SCs have been studied for their prevention and control of animal parasites, diseases, and pests of crops [29]. In China, Japan, South Korea, and Portugal, where PWD is more severe, UAVs or ground spraying have been used to control this disease by spraying chemical SCs that are effective against vector beetles [30,31]. Previous studies showed that the efficiency of the suspension agent sprayed in the forest against vector beetles was about 72.4% [32]. However, there are few reports of applying aerial spraying chemical SCs against PWN. In the present study, the fluopyram SC showed a substantial preventative effect on PWN in the laboratory experiment. After spraying in the field, fluopyram SC showed a significant suppressive effect against PWD within 3 years of application, with a persistence of 3 years and average control efficiency reaching 90.48%.

Moreover, there was an incomplete cleaning of dead wood in sample plot 3, so the efficiency of fluopyram SC to PWD in sample plot 3 was lower than in sample 1 or sample 2, indicating that the integrity of dead wood cleaning could affect the control effect of fluopyram SC. In addition, we also found that the prevention effect of PWD was different when spraying in different months, that application of fluopyram SC later than June can significantly reduce the control effect, whereas early applications of fluopyram SC can achieve a better control efficiency. This is related to the life cycle of the PWN transmitted by vector beetles, whereby the wide spread of PWN occurs from June to September [33]. Therefore, to deploy the efficacy of the fluopyram SC in the field, the best time for application is 1 month before the emergence of beetle adults every year.

In addition, when chemical agents are applied in agriculture and forestry across a large area, their transformation in environmental samples is also the focus of much investigation. For example, several studies have assessed the degradation and metabolism of abamectin and thiacloprid in soil, water, light, and other conditions [34–36], as well as the toxicity and safety of imidacloprid for environmental organisms [37,38]. In the present study, after applying fluopyram SC over a large area, fluopyram residues were detected in soil and water in the non-forest habitat areas between sampling plots. However, we found that these fluopyram residues at 0–200 m, going from the inside to outside of the sampling plot, were almost non-existent 30 days after the application. Even with the small amount of residue detected, the fluopyram residual concentration for soil samples was still significantly lower than the LC_{50} for fluopyram in earthworm (14 d) > 1.00 mg/kg (in dry soil). The residual concentration of water samples 10 m away (0.099 mg/kg) was significantly degraded, which was lower than the LC_{50} for fluopyram in carp (96 h) > 0.98 mg/L. It is reported that fluopyram was degraded chiefly by microorganisms in soil and water and is indeed an easily degraded pesticide [20]. Fluopyram was stable to hydrolysis, with a hydrolysis half-life of more than 1 year at 25 °C [18]. Microbial degradation is the main mode by which fluopyram degrades in soil [18]. According to the Chinese government's standards for the registration and classification of degradation of pesticides in soil, fluopyram should degrade relatively easily [18]. Therefore, applying fluopyram SC in a pine forest is an environmentally friendly practice for pest control.

5. Conclusions

This comprehensive study provides a new, high-efficiency, low-cost control method for the pest management of PWN using fluopyram SC, and the early application of fluopyram SC can effectively reduce the number of dead pine trees in epidemic areas by UAV spraying. This prevention effect can be improved by combining it with cleaning dead wood. In conclusion, fluopyram SC has good control efficiency against PWN with a long duration of efficacy, which together supports its broad application prospects for forest pest control.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f14020338/s1>, Table S1. The information on fluopyram suspension concentrate field application in four sample plots; Table S2. The fluopyram residual concentrations in the pine branches and needles after spraying fluopyram suspension concentrate in different years at four sample plots; Figure S1. Sampling diagram of soil and water samples from the field.

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References

- Kim, B.-N.; Kim, J.H.; Ahn, J.-Y.; Kim, S.; Cho, B.-K.; Kim, Y.-H.; Min, J. A short review of the pinewood nematode, *Bursaphelenchus xylophilus*. *Toxicol. Environ. Health Sci.* **2020**, *12*, 297–304. [[CrossRef](#)]
- Wu, W.; Zhang, Z.; Zheng, L.; Han, C.; Wang, X.; Xu, J.; Wang, X. Research Progress on the Early Monitoring of Pine Wilt Disease Using Hyperspectral Techniques. *Sensors* **2020**, *20*, 3729. [[CrossRef](#)] [[PubMed](#)]
- Hunt, D. Pine wilt disease: A worldwide threat to forest ecosystems. *Nematology* **2009**, *11*, 315–316. [[CrossRef](#)]
- Shi, J.; Luo, Y.-Q.; Xia, N.-B.; Wi, H.-W.; Song, J.-Y. Suggestions on management measures of pine forest ecosystems invaded by *Bursaphelenchus xylophilus*. *For. Stud. China* **2008**, *10*, 45–48. [[CrossRef](#)]
- He, L.X.; Ji, J.; Qiu, X.W.; Zhang, L.P. Occurrence and control measures of pine wood nematode disease in the world. *J. For. Eng.* **2014**, *28*, 8–13.
- Yang, H.; Zhang, R.; Li, Z.; Maienfisch, P.; Xu, X. Design, synthesis and nematocidal activities of trifluorobutene amide derivatives against *Meloidogyne incognita*. *Bioorg. Med. Chem. Lett.* **2021**, *40*, 127917. [[CrossRef](#)]
- James, R.; Tisserat, N.; Todd, T. Prevention of Pine Wilt of Scots Pine (*Pinus sylvestris*) with Systemic Abamectin Injections. *Arboric. Urban For.* **2006**, *32*, 195–201. [[CrossRef](#)]
- Liu, G.; Lin, X.; Xu, S.; Liu, G.; Liu, Z.; Liu, F.; Mu, W. Efficacy of fluopyram as a candidate trunk-injection agent against *Bursaphelenchus xylophilus*. *Eur. J. Plant Pathol.* **2020**, *157*, 403–411. [[CrossRef](#)]
- Avenot, H.F.; Michailides, T.J. Progress in understanding molecular mechanisms and evolution of resistance to succinate dehydrogenase inhibiting (SDHI) fungicides in phytopathogenic fungi. *Crop Prot.* **2010**, *29*, 643–651. [[CrossRef](#)]
- Yang, Z.H.; Tian, H.; Liu, Y.Y. Research development of new-type bactericide-fluopyram. *Mod. Chem. Res.* **2017**, *4*, 103–104.
- Wang, J.; Bradley, C.A.; Stenzel, O.; Pedersen, D.K.; Reuter-Carlson, U.; Chilvers, M.I. Baseline Sensitivity of *Fusarium virguliforme* to Fluopyram Fungicide. *Plant Dis.* **2017**, *101*, 576–582. [[CrossRef](#)]
- Ji, X.; Li, J.; Dong, B.; Zhang, H.; Zhang, S.; Qiao, K. Evaluation of fluopyram for southern root-knot nematode management in tomato production in China. *Crop Prot.* **2019**, *122*, 84–89. [[CrossRef](#)]
- National Forestry and Grassland Administration. Bulletin of the National Forestry and Grassland Administration (No. 6 of 2022) (Pine Wilt Disease Epidemic Areas in 2022). Available online: <https://www.forestry.gov.cn/main/3457/20220318/111403492220586.html> (accessed on 14 May 2022).
- Kobayashi, T.; Sasaki, K.; Mamiya, Y. Fungi associated with *Bursaphelenchus lignicolus*, the pine wood nematode. *J. Jpn. For. Soc.* **1974**, *56*, 136–145. [[CrossRef](#)]

15. Vicente, C.S.L.; Soares, M.; Faria, J.M.S.; Ramos, A.P.; Inácio, M.L. Insights into the Role of Fungi in Pine Wilt Disease. *J. Fungi* **2021**, *7*, 780. [[CrossRef](#)]
16. Chen, Y.; Ye, J.; Wei, C. Effect of pine wood nematode (PWN) infection on phenylpropanes metabolism in *Pinus massoniana* seedlings. *For. Sci.* **2006**, *46*, 73–77.
17. Baermann, G. Eine einfache Methode zur Auffindung von Ankylostomum (Nematoden) larven in Erdproben. *Geneesk. Tijdschr. Voor Ned. Indië* **1917**, *57*, 131–137.
18. Cesarz, S.; Schulz, A.E.; Beugnon, R.; Eisenhauer, N. Testing soil nematode extraction efficiency using different variations of the Baermann-funnel method. *Soil Org.* **2019**, *91*, 61–72. [[CrossRef](#)]
19. Anhui Provincial Forest Pest Control Station. *Control Technology of Pine Wood Nematode Disease*; China Forestry Publishing House: Beijing, China, 2006; Volume DB34/T496-2005.
20. Dong, B.Z. *Transformation Mechanism of the Novel Fungicide Fluopyram in Environment and Biological Toxicity of Its Transformation Products*; University of Science and Technology: Beijing, China, 2016.
21. Lee, S.M.; Chung, Y.J.; Moon, Y.S.; Lee, S.G.; Lee, D.W.; Choo, H.Y.; Lee, C.K. Insecticidal activity and fumigation conditions of several insecticides against Japanese pine sawyer (*Monochamus alternatus*) larvae. *J. Korean For. Soc.* **2003**, *92*, 191–198.
22. Zhao, B.G.; Futai, K.; Sutherland, J.R.; Takeuchi, Y. *Pine Wilt Disease*; Springer: Tokyo, Japan, 2008.
23. Cabrera, J.A.; Menjivar, R.D.; Dababat, A.E.-F.A.; Sikora, R.A. Properties and Nematicide Performance of Avermectins. *J. Phytopathol.* **2013**, *161*, 65–69. [[CrossRef](#)]
24. Takai, K.; Suzuki, T.; Kawazu, K. Development and preventative effect against pine wilt disease of a novel liquid formulation of emamectin benzoate. *Pest Manag. Sci.* **2003**, *59*, 365–370. [[CrossRef](#)]
25. Faske, T.R.; Hurd, K. Sensitivity of *Meloidogyne incognita* and *Rotylenchulus reniformis* to Fluopyram. *J. Nematol.* **2015**, *47*, 316–321. [[PubMed](#)]
26. Veloukas, T.; Karaoglanidis, G.S. Biological activity of the succinate dehydrogenase inhibitor fluopyram against *Botrytis cinerea* and fungal baseline sensitivity. *Pest Manag. Sci.* **2012**, *68*, 858–864. [[CrossRef](#)] [[PubMed](#)]
27. Oka, Y.; Saroya, Y. Effect of fluensulfone and fluopyram on the mobility and infection of second-stage juveniles of *Meloidogyne incognita* and *M. javanica*. *Pest Manag. Sci.* **2019**, *75*, 2095–2106. [[CrossRef](#)] [[PubMed](#)]
28. Hazra, D.K.; Karmakar, R.; Poi, R.; Bhattacharya, S.; Mondal, S. Recent advances in pesticide formulations for eco-friendly and sustainable vegetable pest management: A review. *Arch. Agric. Environ. Sci.* **2017**, *2*, 232–237.
29. Cai, J.Z.; Li, C.H.; Liu, S.C.; Bai, Z.Y. Effect and safety of ivermectin dry suspension agent against sheep parasites. *Vet. Sci. China* **2006**, *4*, 296–300.
30. Sousa, E.; Rodrigues, J.M.; Bonifácio, L.; Naves, P.M.; Rodrigues, A. Management and control of the pine wood nematode, *Bursaphelenchus xylophilus*, in Portugal. In *Nematodes: Morphology, Functions and Management Strategies*; Boeri, F., Chung, J.A., Eds.; Nova Science Publishers, Inc.: Hauppauge, NY, USA, 2011; pp. 157–178.
31. Mamiya, Y. History of pine wilt disease in Japan. *J. Nematol.* **1988**, *20*, 219–226.
32. Sheng, C.S. Experimental study on the control of *Monochamus alternatus* Hope by spraying cypermethrin and imidacloprid by aircraft. *Anhui For. Sci. Technol.* **2006**, *1*, 6–7+10.
33. Togashi, K. Population density of *Monochamus alternatus* adults (Coleoptera: Cerambycidae) and incidence of pine wilt disease caused by *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae). *Popul. Ecol.* **1988**, *30*, 177–192. [[CrossRef](#)]
34. Torabi, E.; Jahromi, K.T.; Homayoonzadeh, M.; Torshiz, A.O.; Tavakoli, E. Residue kinetics of neonicotinoids and abamectin in pistachio nuts under field conditions: Model selection, effects of multiple sprayings, and risk assessment. *Environ. Sci. Pollut. Res.* **2022**, *29*, 2598–2612. [[CrossRef](#)]
35. Gupta, S.; Gajbhiye, V.T.; Gupta, R.K. Effect of Light on the Degradation of Two Neonicotinoids viz Acetamiprid and Thiacloprid in Soil. *Bull. Environ. Contam. Toxicol.* **2008**, *81*, 185–189. [[CrossRef](#)]
36. Englert, D.; Bakanov, N.; Zubrod, J.P.; Schulz, R.; Bundschuh, M. Modeling Remobilization of Neonicotinoid Residues from Tree Foliage in Streams—A Relevant Exposure Pathway in Risk Assessment? *Environ. Sci. Technol.* **2017**, *51*, 1785–1794. [[CrossRef](#)]
37. Tattar, T.; Dotson, J.; Ruizzo, M.; Steward, V.B. Translocation of Imidacloprid in Three Tree Species when Trunk- And Soil-Injected. *Arboric. Urban For.* **1998**, *24*, 54–56. [[CrossRef](#)]
38. Smit, C.E.; Posthuma-Doodeman, C.J.A.M.; Van Vlaardingen, P.L.A.; De Jong, F.M.W. Ecotoxicity of Imidacloprid to Aquatic Organisms: Derivation of Water Quality Standards for Peak and Long-Term Exposure. *Hum. Ecol. Risk Assess.* **2015**, *21*, 1608–1630. [[CrossRef](#)]

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