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Hot Water Treatment as a Quarantine Measure for Controlling *Pratylenchus penetrans* Cobb in *Syngonium podophyllum* Schott and *Perilla frutescens* Britton

Ga-Eul Lim [1], Moon-Sun Heo [2] and Min-Goo Park [1,*]

1 Department of Bioenvironmental Chemistry, Jeonbuk National University, Jeonju 54896, Republic of Korea; na9412@naver.com
2 Research and Development Division, Goyang-Si Agricultural Technology Center, Goyang 10563, Republic of Korea; forology@korea.kr
* Correspondence: pmg@korea.kr

Abstract: *Syngonium podophyllum* Schott plants are increasingly being imported in Korea due to their ability to purify indoor air. Root lesion nematodes, *Pratylenchus* spp., are the most frequently occurring nematodes associated with *S. podophyllum*, leading to the disposal of plants or their return to the country of origin, due to inadequate phytosanitary treatments. We evaluated the effectiveness of hot water treatment in controlling *Pratylenchus penetrans* Cobb, present in *S. podophyllum* and *Perilla frutescens* Britton. The mortality rate ($LT_{99\%}$: the time at which 99% lethality is achieved) of *P. penetrans* at 44 to 52 °C water and treatment-caused plant damage were evaluated in vitro and in vivo. The in vitro test showed the LT_{99} at 44, 46, 48, 50, and 52 °C was 119.1, 27.0, 14.70, 1.40, and 0.48 min, respectively. The LT_{99} of hot water treatment on the nematodes infecting both plants was 18.4 to 1.7 min at 46 to 50 °C. *S. podophyllum* was undamaged at 30 days after treatment with 49 and 51 °C water for 30 min. This study demonstrates that hot water treatment is a potential phytosanitary disinfection method for *Pratylenchus* spp. infecting foliage plants, which can accelerate their trade by reducing incidents of disposal or return to the country of origin.

Keywords: foliage plant; hot water immersion; nematode; phytosanitary treatment; root lesion nematodes

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1. Introduction

Seedlings imported into the Republic of Korea occupy a large proportion of quarantine facilities. Among them, 316,000 individual *Syngonium podophyllum* Schott plants (Araceae, arrowhead vine) were imported from 2021 to 2023 [1]. Specifically, the import of *S. podophyllum* almost doubled from 2018 to 2020, from an initial 140,940 units [1]. This is because *S. podophyllum* is an attractive indoor ornamental plant due to its excellent ability to detoxify formaldehydes in the air [2].

Nematode infestations have become a significant concern when inspecting imported seedlings, including *S. podophyllum*. Notably, *Pratylenchus* root lesion nematodes were detected in a total of 654 cases, making them the most frequently occurring nematodes discovered during the period from 2021 to 2023 [1]. The *Pratylenchus* spp. detected include *P. brachyurus* Godfrey, *P. coffeae* Zimmermann, *P. fallax* Seinhost, *P. neglectus* Rensch, *P. penetrans* Cobb, and *P. projectus* Jenkins. Among them, *P. penetrans* ranked second among the detected nematodes, with 147 cases [1].

Pratylenchus penetrans exhibits a broad host range, affecting approximately 400 plant species [3], including ornamental plants such as roses and daylilies [4]. While this nematode is globally distributed, some countries categorize it as a pest requiring quarantine management. Paraguay has designated it as grade A1, Jordan as grade A2, and Mexico has classified it as a quarantine pest [5]. *Pratylenchus penetrans* is a nematode of major importance in Korea, as it causes significant economic damage in cultivated crops such as perilla and strawberries. In particular, the infection rate of *P. penetrans* was found to be over 55% in Geumsan and Miryang, which are the main areas for producing leaf perilla seeds. This high infection rate was especially notable during the continuous cropping period, leading to inhibited leaf growth and production [6]. Despite the use of nematicides on the host plants, complete eradication is challenging once soils are infested [7]. Therefore, it is necessary to implement effective disinfection methods to prevent *P. penetrans* infestations, and to maintain the potential for exporting these plants outside of Korea. Additionally, for seedlings to be accepted into Korea, phytosanitary treatments would be required to eliminate plant infestations caused by *Pratylenchus* spp.

The Animal and Plant Quarantine Agency in Korea mandates adherence to the International Plant Protection Convention (IPPC), requiring a Probit 9 efficacy for invasive pests in imported plants, which indicates a 99.9968% mortality rate through quarantine treatments [8]. Nematodes are no exception to this standard.

Hot water treatment has been recognized as an effective control method against nematodes in seedlings worldwide [9]. For the genus *Pratylenchus*, treatment conditions vary based on the type of plant, such as grapevine, potato, *Allium hookeri* Thwaites, or ginger, but effective control of nematodes has been achieved at temperatures between 43 °C and 50 °C for 1 min to 1 h [10–14]. However, little research has been conducted on controlling nematodes in foliage plants, which are vulnerable to heat, and there is no prescribed treatment method to control nematodes in the plants found in Korea's quarantine inspection according to Phytosanitary disinfestation guidelines [15]. This leads to the disposal or return of infected consignments to the country of origin. The disposal and return of imported foliage plants cause an economic loss of approximately 100 thousand US dollars per 40 ft container, increasing the distribution costs of the plants into the country. Thus, developing an effective phytosanitary treatment for these plants is an urgent issue.

In this study, we evaluated the feasibility of hot water treatment for controlling *P. penetrans* in *S. podophyllum* and *Perilla frutescens* Britton, which were selected as representative foliage plants and major hosts of *P. penetrans* in Korea, respectively. Specifically, we (1) determined the efficacy of hot water treatment on *P. penetrans* in vitro; (2) evaluated the effect of temperature increase in relation to the diameter and filling ratio of *Syngonium* roots; (3) conducted efficacy tests of hot water treatment on *P. penetrans* infecting *S. podophyllum* and *P. frutescens* as an in vivo test, based on the results of (1) and (2); and (4) assessed the effect of heat treatment on the quality of *S. podophyllum*.

2. Materials and Methods

2.1. Nematodes

Pratylenchus penetrans were provided by the Crop Protection Division of the Rural Development Administration in Wanju, Republic of Korea, which were propagated from *P. frutescens* and *S. podophyllum*. The nematodes were identified by a specialist in the division. *Pratylenchus penetrans* is characterized by a slender, cylindrical body with a distinct lip region and a feeding stylet. It is dioecious, with males having a curved tail and females having a straight tail with a vulva. Nematodes in infected hosts were separated in an environment of 25 ± 1 °C using a Baermann funnel [16]. The separated nematodes were collected, and their species and density were determined under an inverted microscope. The life stages of the nematodes used in this study were a mixture of adults and juveniles.

2.2. Efficacy of Hot Water Treatment on P. penetrans in an In Vitro Test

In order to evaluate the efficacy of hot water treatment on *P. penetrans*, the temperature of the 50 L constant temperature water bath (Ian Science, C-AWB1, Seoul, Republic of Korea) was set at 44, 46, 48, 50, and 52 °C. Adult female nematodes were selected after separation from *P. frutescens*. These nematodes were suspended in water at a concentration of 50 to 100 individuals per 1 mL and transferred to test tubes (25 mm [d] × 20 mm [h], UNI B&C, Goyang, Republic of Korea) with a perforated bottom with a 500-mesh sieve for immersion with a 1 mL micropipette; no water was left in the sieve. The nematodes were injected into the test tube, and it was immersed in the tank and maintained for 10 s, 30 s, and 1, 3, 5, 10, 20, 40, and 60 min each, after the temperature inside the test tube had reached the target temperature. Separate replicates were prepared for each of the nine submersion durations at each temperature. However, when shorter submersion durations at higher temperatures killed 100% of the nematodes, we did not test the longer time intervals, such as 20 to 60 min. Water temperature was recorded using a multi-channel thermometer (LUTRON, BTM-4208SD, Taipei, Taiwan). After the treatment, the nematodes were transferred to Petri dishes with distilled water and stored at room temperature (25 °C).

The survival of the nematodes was assessed 24 h after hot water treatment. They were considered deceased when they showed no response to stimuli. All treatments were repeated in triplicate.

2.3. Effect of Temperature Increase Relative to the Diameters and Filling Ratio of S. podophyllum Roots

To monitor temperature within the roots of *S. podophyllum*, a multi-channel thermometer probe was inserted into the center of *S. podophyllum* roots. The initial temperature of the roots was set to 19 °C. The immersion was conducted in a water bath with a volume of 20.25 L, maintaining a water temperature of 50 °C. The temperatures of the roots were monitored at three points in each *S. podophyllum.* The three points were the thinnest, with a medium diameter, and the thickest part of each *S. podophyllum* root. Root diameters were measured using calipers (MITUTOYO, CD-15APX, Tokyo, Japan). Ramping time was recorded from an initial temperature of 19 °C to the target temperature of 50 °C on each root after it was immersed in the water bath.

To evaluate the effect of temperature increase depending on the filling ratio, *S. podophyllum* roots were designated as 0.6, 1.19, 1.28, 1.88, 2.07, 2.47, 2.67, and 4.37 g/L of the filling ratio. The ratio was defined as the root mass/volume of water in the bath (g/L). The temperature was monitored in the thickest part of each *S. podophyllum* root. All trials were replicated three times.

2.4. Efficacy of Hot Water Treatment on P. penetrans Infecting S. podophyllum and P. frutescens in an In Vivo Test

Pratylenchus penetrans was cultured in *S. podophyllum* and *P. frutescens* for nearly 6 months, from April to October 2023, in a greenhouse at the Rural Development Administration. The nematodes in the plants were counted prior to treatment, and those with an infestation of over 150 nematodes were treated. One plant was used for each treatment.

The temperature of the water bath was set to 46, 48, or 50 °C for *P. frutescens*, and 50 or 52 °C for *S. podophyllum*. After immersing the plants, the internal root temperature was maintained for 1, 3, 10, 20, and 40 min, starting from the point when the target temperature was reached. The temperature and duration were set based on the result of the in vitro test.

Following immersion, the roots were air-cooled for 1 h before the nematodes were extracted using the Baermann funnel method, allowing them to leach for over 24 h. After that, their mortality was assessed. All nematodes separated using the Baermann funnel method were considered surviving individuals from the hot water immersion treatment, regardless of their responsiveness to stimuli. The mortality rate was calculated by comparing the average number of nematodes separated from the untreated group with those from the treated groups. All trials were replicated three times.

2.5. Effect of Hot Water Treatment on the Quality of S. podophyllum

To evaluate the effect of hot water treatment on the quality of *S. podophyllum*, the plants were immersed in hot water for 0, 1, 5, 10, 15, and 30 min at 49 or 51 °C. One plant was used per treatment. The water temperature was raised by 1 °C above the target temperature of 48 and 50 °C to account for temperature deviation. After treatment, the plants were replanted in horticultural bed soil. Changes in the mass of the entire plant were recorded 30 days after replanting. The control group (0 min immersion) was subjected to the same process as the treated groups, with the exception of hot water treatment. All trials were replicated three times.

2.6. Data Analysis

The time-response effects of heat water treatment on *P. penetrans* in the in vitro and in vivo tests were estimated using Probit analysis [17], based on a computer program written by Ge Le Pattourel, Imperial College, London, UK and adopted by Don-Pedro (1989) [18]. In the probit parameters, 'slope' indicates the relationship between the time at a specific temperature and the lethality of *P. penetrans*. '*df*' represents the degrees of freedom associated with the number of cases treated. $LT_{50\%}$ and $LT_{99\%}$ indicate the time at which 50% and 99% lethality of the nematodes is achieved, respectively. Simple regression analysis was performed to reveal the monotonically increasing trend of time according to the diameter and filling ratio of the plant's root (SPSS ver. 23). Differences in the quality measurements of *S. podophyllum* among the 0-, 1-, 5-, 10-, 15-, and 30-minute treatment groups on the target temperature were estimated using ANOVA (SPSS ver. 23).

3. Results

3.1. Efficacy of Hot Water Treatment on P. penetrans in In Vitro Tests

The LT_{99} values of hot water treatment on *P. penetrans* were 119.13 min at 44 °C, 27.03 min at 46 °C, 14.70 min at 48 °C, 1.40 min at 50 °C, and 0.48 min at 52 °C, respectively (Table 1). In the 44–48 °C treatments, a mortality rate of less than 40% was observed from 10 s to 1 min. In contrast, temperatures of 50 °C and 52 °C exhibited mortality rates exceeding 80% even at 10 s, reaching 100% mortality after 3 min (Table 1, Figure 1).

Table 1. Efficacy of hot water treatment at different temperatures on *Pratylenchus penetrans* Cobb mortality in a small-scale in vitro test (50 L).

Temp. (°C)	No. of *P. penetrans*	$LT_{50\%}$ [a] (95% CI, min)	$LT_{99\%}$ [a] (95% CI, min)	Slope ± SE [b]	*df* [c]	X^2 [d]
44	1008	3.31 (2.41–4.48)	119.13 (69.22–245.43)	1.49 ± 0.12	19	166.07
46	1399	2.68 (2.09–3.36)	27.03 (17.20–54.80)	2.32 ± 0.27	19	76.51
48	1649	1.08 (0.91–1.29)	14.70 (10.48–22.59)	2.05 ± 0.13	25	239.72
50	799	0.03 (0.01–0.06)	1.40 (0.90–3.16)	1.43 ± 0.24	13	34.66
52	386	0.04 (0.01–0.08)	0.48 (0.31–5.48)	2.09 ± 0.79	13	6.95

[a] $LT_{50\%}$ and $LT_{99\%}$ values with 95% CIs indicate the time at which 50% and 99% lethality of the nematodes is achieved, with a 95% confidence interval, respectively. [b] Slope indicates the relationship between the time at a specific temperature and the lethality of *P. penetrans*. SE: standard error. [c] Degree of freedom. [d] X^2 based on pooling of data with low expectation.

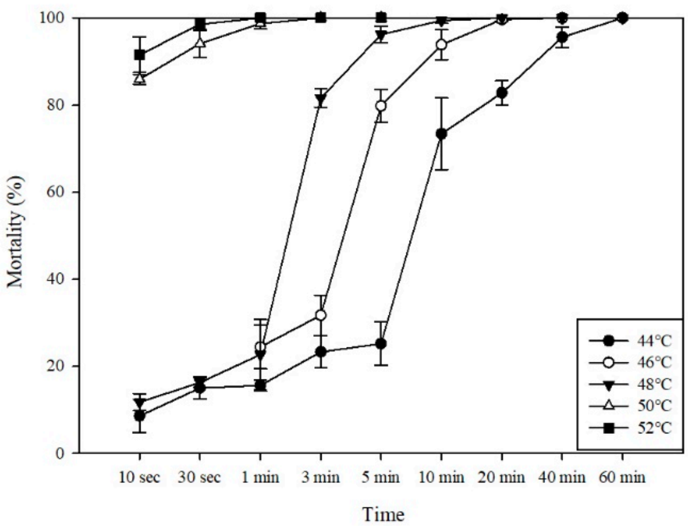

Figure 1. Mortality trends of *Pratylenchus penetrans* Cobb relative to the duration of exposure at each target temperature in a hot water in vitro test.

3.2. Effect of Temperature Increase in Relation to Diameter and Filling Ratio of Syngonium Roots

The increase in the root diameter of *S. podophyllum* increased the time required to reach the target temperature, with a trend equation between the parameters of y = 13.39x − 26.111 (R^2 = 0.8029) (Figure 2a). The largest root diameter used in this experiment was 12 mm. It took less than 3 min to reach the target temperature of 50 °C after immersion.

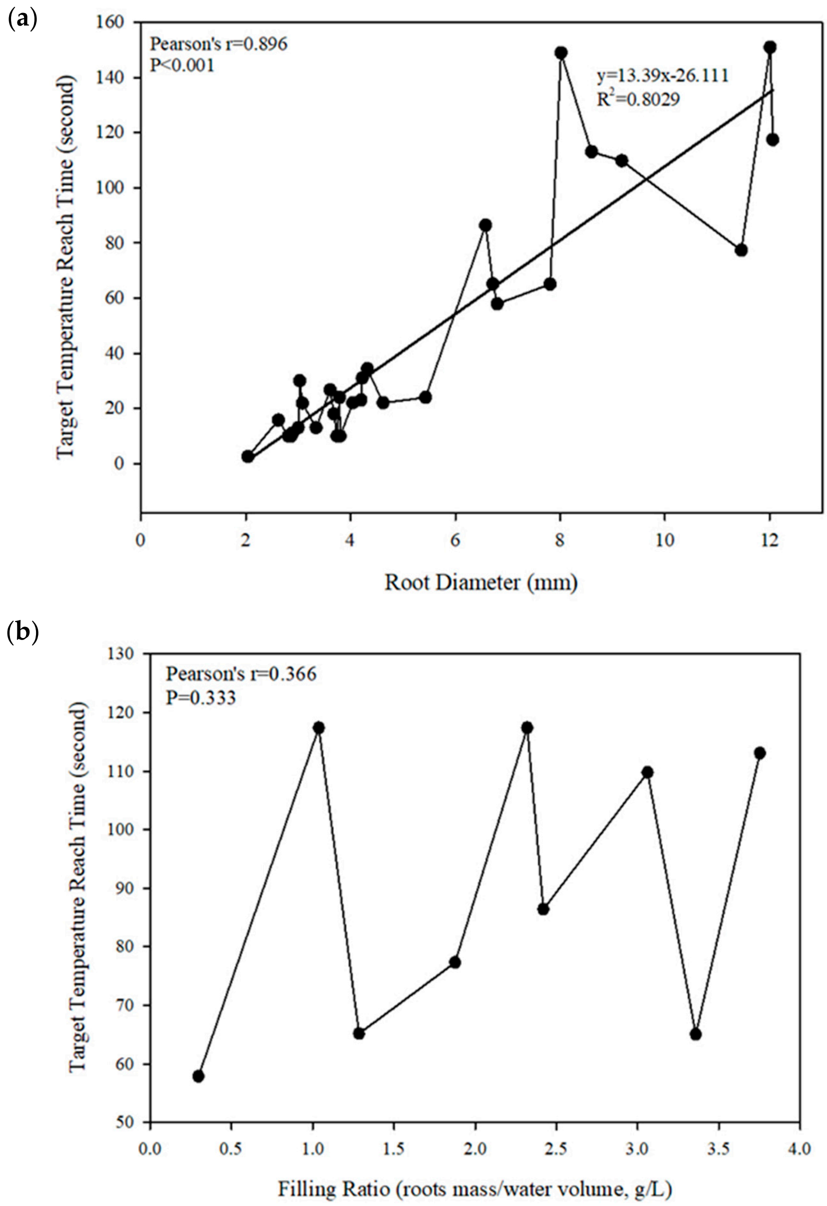

Figure 2. Variation in the time taken for *Syngonium podophyllum* Schottt roots to reach the target temperature, depending on the root diameter (**a**) and filling ratio (**b**).

The temperature increase showed no significant relationships to the filling ratios at *S. podophyllum*. The *p*-values for the filling ratio were 0.333 for the thick diameter group, indicating the lack of a significant relationship (Figure 2b).

3.3. Efficacy of Hot Water Treatment on P. penetrans Infecting S. podophyllum and P. frutescens

The LT_{99} value of *P. penetrans* infecting *S. podophyllum* was 2.67 min at 50 °C, and complete mortality was observed after treatment for 1 min at 52 °C. For *P. penetrans* infecting *P. frutescens*, LT_{99} values of 18.42, 18.59, and 1.68 min were obtained for treatments at 46, 48, and 50 °C, respectively (Table 2). Considering that the LT_{99} values in the in vitro test were 27.03, 14.70, 1.40, and 0.48 min at 46, 48, 50, and 52 °C, respectively, the results of this in vivo test were comparable to those of the in vitro test.

Table 2. Efficacy of hot water treatment at different temperatures on *Pratylenchus penetrans* Cobb mortality in the roots of *Perilla frutescens* Britton and *Syngonium podophyllum* Schott.

Plant	Temp. (°C)	$LT_{50\%}$ [a] (95% CI, min)	$LT_{99\%}$ [a] (95% CI, min)	Slope ± SE [b]	*df* [c]	X^2 [d]
P. frustescens	46	0.77 (0.50–1.05)	18.42 (11.82–36.50)	1.69 ± 0.18	13	84.92
	48	0.90 (0.49–1.30)	18.59 (10.34–55.91)	1.77 ± 0.27	13	42.56
	50	0.03 (0.01–0.08)	1.68 (1.32–2.19)	1.32 ± 0.24	10	31.00
S. podophyllum	50	0.01 (0.01–0.04)	2.67(1.69–4.95)	0.91 ± 0.21	13	18.04
	52	<1.00	<1.00	-	-	-

[a] $LT_{50\%}$ and $LT_{99\%}$ values with 95% CIs indicate the time at which 50% and 99% lethality of the nematodes is achieved, with a 95% confidence interval, respectively. [b] Slope indicates the relationship between the time at a certain temperature and the lethality of *P. penetrans*. SE: standard error. [c] Degree of freedom. [d] X^2 based on pooling of data with low expectation.

Mortality of *P. penetrans* exceeding 95% was observed after 1 min at 50 °C or more for both host plants, although *P. penetrans* mortality above 50% was observed for both the 46 and 48 °C treatments in *P. frutescens* (Figure 3).

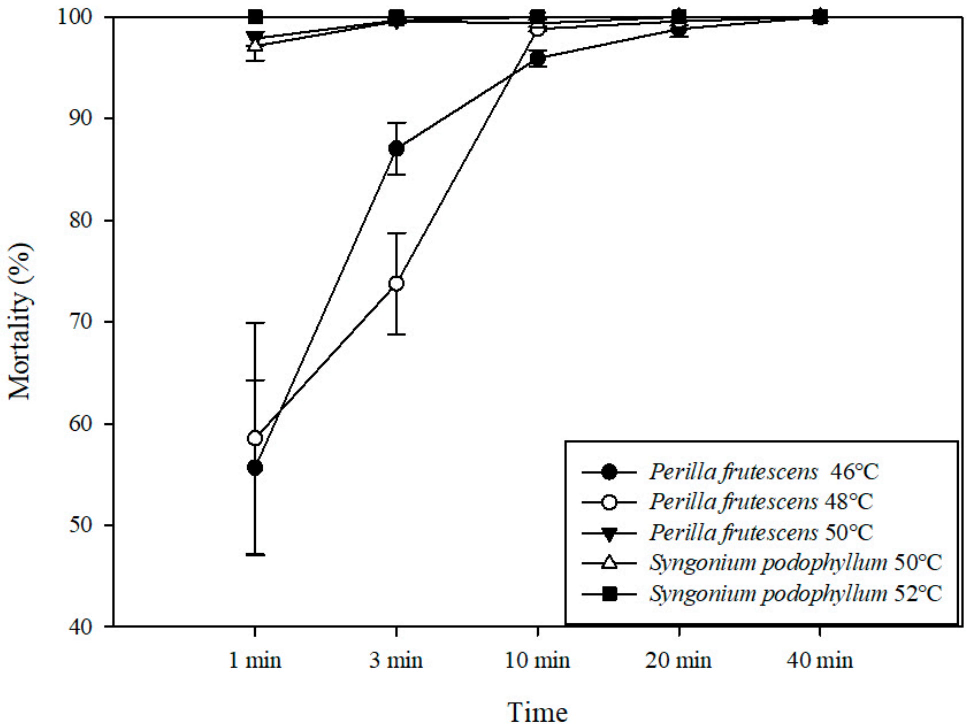

Figure 3. Trend of increasing *Pratylenchus penetrans* Cobb mortality in infected *Perilla frutescens* Britton and *Syngonium podophyllum* Schott roots following hot water treatment at different temperatures.

The LT_{99} values at 50 °C in *S. podophyllum* and *P. frutescens* were 1.68 min (confidence level, CL 1.32–2.19) and 2.67 min (CL 1.69–4.95), respectively, which did not differ significantly. This indicates there was no difference in the susceptibility of the two host plants to hot water treatment against nematodes (Table 2). Considering the LT_{99} of 1.40 (CL 0.90–3.16) at 50 °C in the in vitro trials (Table 1), we confirmed that similar efficacy was achieved in the in vivo condition where nematodes had infected plants.

3.4. Effect of Hot Water Treatment on Quality of *S. podophyllum*

At 30 days after heat treatment of *S. podophyllum*, there was no heat-induced damage and normal growth was confirmed (Figures 4 and 5). When comparing the growth rates before and after treatment for all conditions, including the highest temperature and longest duration of 51 °C for 30 min, there were no statistically significant differences in weight due to heat stress ($p > 0.05$ in Table 3).

Figure 4. Effect of hot water treatment on *Syngonium podophyllum* Schott. C: 0 min immersion; 1, 2, and 3 were exposed to 49 °C for 30 min in the water bath, after which the whole plant was cultured for 30 days.

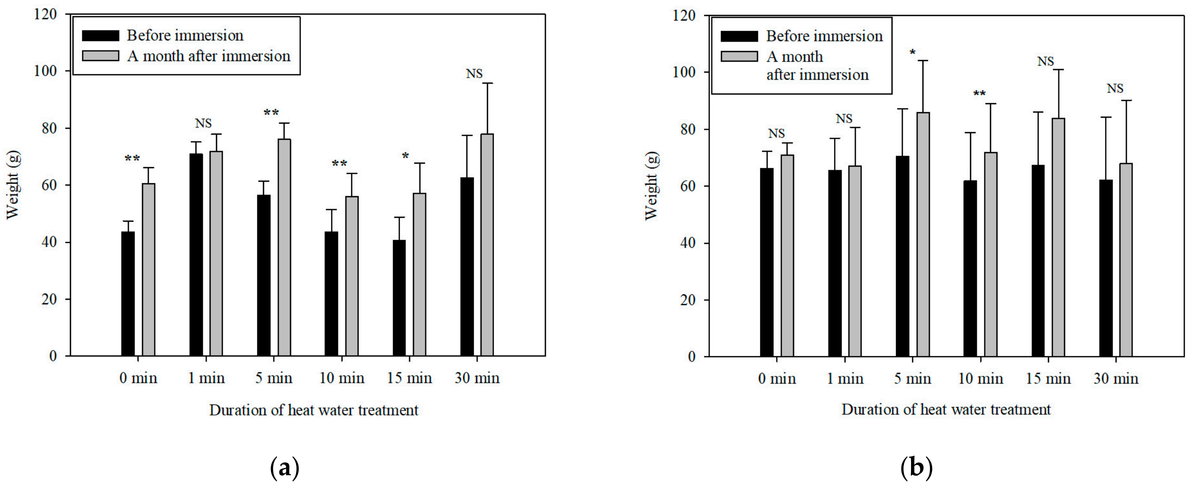

Figure 5. Increasing trends of *Syngonium podophyllum* Schott mass 30 days after hot water treatment when immersed for 0, 1, 5, 10, 15, and 30 min at 49 °C (**a**) and at 51 °C (**b**). * $p < 0.05$, ** $p < 0.01$, and NS: not significant.

Table 3. The increasing rate (%) of *Syngonium podophyllum* Schott mass with growing time following exposure to hot water at 49 °C and 51 °C.

Temp. (°C)	Treatment Time						F	p
	0 min	1 min	5 min	10 min	15 min	30 min		
49 °C	38.6 ± 6.9	18.7 ± 6.4	35.3 ± 4.0	31.0 ± 6.6	41.7 ± 4.1	25.7 ± 7.3	2.896	0.053
51 °C	9.6 ± 8.3	3.3 ± 2.3	24.2 ± 5.76	20.1 ± 7.2	33.0 ± 15.2	17.5 ± 12.6	1.180	0.367

Values are represented as mean ± SE (n = 3), except for 0 min (n = 5). They indicate an increasing rate (%) of *S. podophyllum* mass over a 30-day cultivation period after the plant was treated with hot water for varying amounts of time.

4. Discussion

The application of hot water treatment for controlling various plant parasitic nematodes, such as root lesion nematodes [10,11,13,14,19], burrowing nematodes [9,20–23], or root-knot nematodes [12,19,24–27] has been studied. This has been investigated for elimination of the nematodes in bananas [20,28], potato tubers [11], flower bulbs [27,29], vegetative seeds [30], rootstocks of roses [31], and grapevines [14,24,26]. However, determining the temperature and duration of hot water treatment for *P. penetrans* in the roots of *S. podophyllum* and *P. frutescens* during quarantine was determined for the first time. Hot water treatment proved effective against the quarantine pest *P. penetrans* in an in vitro test. At temperatures of 50 °C and 52 °C, mortality rates surpassed 80% within 10 s, achieving complete mortality within 3 min. The nematodes infesting the roots of *S. podophyllum* and *P. frutescens* were successfully controlled and the treatment caused no thermal damage to the plants.

According to Arcinas's study on *Radopholus similis* Cobb in an in vitro test, the LT_{99} value at 47 °C was 5.1 min [32]. *R. similis* has been listed as a prohibited pest for regulation in Korea, as it is a nematode of major importance that damages various plants worldwide. In our study, the LT_{99} of *P. penetrans* at 46, 48, and 50 °C was 27.0, 14.7, and 1.4 min, respectively. Comparing the two studies' results, *P. penetrans* appears to exhibit higher heat tolerance compared to *R. similis*. Therefore, the temperature and exposure duration used for *P. penetrans* control can also be used for controlling *R. similis*.

In in vivo tests for *Pratylenchus* spp. in previous studies, hot water treatment at 49 °C for 10 min was effective for controlling *P. coffea* in *A. hookeri* [12], whereas *P. penetrans* in young apple trees was controlled when exposed to water at 46 to 47 °C for 30 min [33]. In addition, the temperature range to control *P. penetrans* is mainly between 43 and 48 °C, with exposure for 30 min to 1 h [10]. Considering that LT_{99} on *P. penetrans* in *P. frutescens* at 46 and 48 °C was 18.4 and 18.6 min, respectively, in our study, we can confirm that our results are consistent with previous studies. Susceptibility of *P. penetrans* to hot water treatment also did not differ significantly between *S. podophyllum* and *P. frutescens* (Table 2). Thus, the temperature and time could potentially be applied to different foliage plants, such as *Anthurium* and *Caladium*, which are also known to improve human health by cleaning the air.

When the results of the in vitro and in vivo tests were compared, the 95% confidence limits of the LT_{99} values at 48 °C were 10.48–22.59 and 10.34–55.91 min, respectively, whereas at 50 °C, they were 0.90–3.16 and 1.32–2.19 min (Tables 1 and 2). There were no significant differences between them, suggesting that nematode infestations of plants did not affect their heat sensitivity.

This can be attributed to the rapid ramping speed of the temperature inside the roots. We investigated the heat conductivity into irregularly shaped *S. podophyllum* roots based on different root thicknesses. We found that the time taken to reach the target temperature of 50 °C within the roots increased as the root thickness increased, and that ramping took less than 3 min when the root diameter ranged from 2 to 12 mm (Figure 2). The increasing trend equation, $y = 13.39x - 26.111$ ($R^2 = 0.8029$), calculated in this study can be utilized for predicting the time to reach the target temperature depending on the root diameter.

Hot water treatment did not cause heat damage in *A. hookeri* at 48 °C when exposed for 5 to 30 min and at 49 °C for 5 to 10 min [7]. In this study, *S. podophyllum* treated at 49 and 51 °C for 30 min had no statistically significant difference in the weight gain ratio compared to the control ($p > 0.05$). This indicates that *S. podophyllum* is more heat tolerant than *A. hookeri*. We can thus confirm that the impact of hot water treatment may vary depending on the variety and plant species [34].

To develop appropriate phytosanitary treatment measures to control new pests, several tests are necessary, including efficacy data under operational as well as laboratory conditions [8]. Future research should be carried out to evaluate the efficacy and damage that the nematodes cause to the plants at a commercial scale. Moreover, more nematode species should be considered for different plants with larger root diameters to further develop this treatment as a quarantine process. Nevertheless, this study identified the temperature and time that can effectively control a newly identified pest, *P. penetrans*, in two newly imported plants, *S. podophyllum* and *P. frutescens*. The applicability of the results from this study, including the ramping speed of the root temperature and the lack of damage from hot water treatment, was also confirmed. The research will help set a standard for phytosanitary treatment using hot water treatment and will further accelerate the trade of foliage plants including *S. podophyllum* by reducing economic losses from disposal or return of infected plants to the county of origin.

Author Contributions: Project administration, supervision, funding acquisition, review and editing, M.-G.P.; investigation, methodology and formal analysis, M.-S.H.; formal analysis, writing—original draft preparation, validation and data curation, G.-E.L. All authors have read and agreed to the published version of the manuscript.

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

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