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Refinement of Hot Water Treatment for Management of *Aphelenchoides fragariae* in Strawberry[1]

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Abstract: The effects of hot water treatments on a California population of the foliar nematode, *Aphelenchoides fragariae,* and on five strawberry cultivars ('Chandler', 'Douglas', 'Fern', 'Pajaro', and 'Selva') were assessed in laboratory and greenhouse tests. Nematodes extracted from fern leaves were placed in water maintained at 44.4, 46.1, 47.7, or 49.4 C for different time periods. Exposure periods of 15, 5, 4, and 2 minutes were required to produce 100% mortality at 44.4, 46.1, 47.7, and 49.4 C, respectively. In a water bath, 4 minutes were required for strawberry crowns initially at 25 C to equilibrate with temperatures ranging from 44.4–54.4 C. The maximum exposure periods that did not significantly reduce subsequent plant growth and flowering were 30, 15, and 10 minutes, at 44.4, 46.1, and 47.7 C, respectively. Survival of Selva was lower ($P = 0.05$) than for the other cultivars. Treatment at 49.4 C for 5 minutes significantly reduced plant growth and flowering of all cultivars. The minimum–maximum exposure periods that killed *A. fragariae* without damaging the cultivars tested were 20–30 minutes at 44.4 C, 10–15 at 46.1 C, or 8–10 at 47.7 C.

Key words: Aphelenchoides fragariae, foliar nematode, *Fragaria chiloensis,* hot water treatment, nematode, strawberry.

Strawberry (*Fragaria chiloensis*) is an important crop in California. In 1990, fruit production was 557 million kg on 8,500 hectares with a value of 523 million dollars (5). Where the foliar nematode, *Aphelenchoides fragariae,* is present, yields are reduced. This nematode is typically found in the crowns of strawberry plants, where it feeds on leaf and flower buds (3,7,15).

Hot water treatment of dormant crowns has been available for management of *A. fragariae* for more than 50 years but is not widely used in California. Unfortunately, recommended exposure periods and temperatures vary (4,6,8–10,12,13,16), and differences in sensitivity have been reported for populations of *A. fragariae* (6). California growers are concerned that new strawberry cultivars may be more sensitive than those on which treatments were developed initially. Our experiments were designed to i) determine the time required for various temperatures to kill a California population of *A. fragariae* and ii) to evaluate the effects of treatment of dormant crowns on subsequent plant growth, runner production, and flower production of five strawberry cultivars.

Materials and Methods

Hot water bath and strawberry plants: A 21-liter constant-temperature water bath was constructed from a 32-liter polyethylene ice chest and a thermostatically controlled immersion heater with a circulating pump (Mgw Lauda, Model T-1, Germany). Temperature in the water bath was controlled within 0.1 C and monitored with type T (copper-constantan) thermocouples (Omega Engineering, Inc., Stamford, CT) connected to a chart recorder (Houston Omniscribe Chart Recorder Model B5237-5, Western Scientific Associates, Danville, CA) via an automatic signal scanner (Omega Engineering) and digital temperature meter (Omega Model 680) with an internal ice-point reference.

Dormant crowns of the strawberry cultivars 'Chandler', 'Douglas', 'Fern', 'Pajaro', and 'Selva' were obtained each year for 3 years from a commercial nursery in Shasta County, California, and stored at 0 C for up to 5 months until needed for experiments.

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Effects of treatment on nematode mortality: A population of *A. fragariae* known to infest strawberry was reared under monoxenic conditions at 25 C in bird's-nest fern, *Asplenium nidus*. Nematodes were extracted from leaves placed in a mist chamber (1) for 12 hours. Extracted nematodes were rinsed in tap water, collected on a 25-μm-pore sieve, and transferred to aerated tap water at 15 C in which they were held for up to 24 hours before use in experiments.

Three replicates of each of the following temperature–time combinations were tested: 44.4 C for 5, 10, 15, 20, 25, and 30 minutes; 46.1 C for 2, 3, 4, 5, 10, 15, 20, 25, and 30 minutes; 47.7 C for 2, 3, 4, 5, 10, and 15 minutes; and 49.5 C for 2, 3, 4, 5, and 10 minutes. The experiment was conducted three times.

Test tubes (25 ml) containing 9 ml tap water were placed in the hot water bath and allowed to equilibrate. Following equilibration, one ml of nematode suspension containing approximately 50 nematodes with approximately 15% juveniles, 5% males, and 80% females, was added to each test tube. The control treatment was room temperature (25 C). Test tubes were removed from the water bath at each time interval and placed immediately into a water bath at 25 C. After cooling, tubes were removed from the 25 C water bath and allowed to sit in the laboratory (25 C) for 24 to 48 hours. The numbers of live and dead nematodes in each tube were then counted. Motionless nematodes were touched with a dissecting needle, and those that did not move were counted as dead (17). The average mortality of nematodes from controls held at room temperature for 48 hours was used as a correction factor for mortality that occurred during treatment. The percentage mortality caused by treatment was calculated from the following formula: $100 \times [D_t - (N_t \times D_n)]/[N_t - (N_t \times D_n)]$, where D_t is the total number of dead nematodes, N_t is the total number of live plus dead nematodes, and D_n is the average percentage mortality of the nematodes in the control. An angular transformation was performed on mortality data prior to analysis of variance (ANOVA) (11). Preliminary statistical analysis indicated that there were no differences ($P = 0.05$) among the three experiments. Therefore, data from the three experiments at each temperature–time combination were pooled, and Bonferroni's test was performed on the pooled data (14).

Effect of initial strawberry crown temperature on time required to reach a higher temperature: Chandler crowns were placed in tap water (25 C) to thaw and equilibrate. Temperature probes were inserted into the centers (five replicate crowns per treatment). The crowns were immersed in hot water baths with constant temperatures of 44.4, 46.1, 47.7, 49.4, 51.1, 52.7, or 54.4 C, and the time required to raise the crown temperature to that of the bath was determined. Results were subjected to linear regression and Bonferroni's test (14).

Effect of treatment on subsequent survival and runner and flower production: Strawberry crowns were placed in tap water to thaw prior to treatment. The five cultivars were exposed to water maintained at 44.4, 46.1, 47.7, or 49.4 C for 5, 10, 15, 20, 25, or 30 minutes. Immediately after treatment, crowns were transferred to tap water for rapid cooling and then planted in 400-cm^3 styrofoam cups containing steam sterilized sand and loam (2:1) mix. Four replicates of two plants each were grown in a greenhouse for each treatment. Eight plants without treatment served as controls. The experiments were performed with all time and temperature combinations randomized separately for each cultivar and conducted in 1988, 1989, and 1990 (except cultivar Douglas, which was not available for testing in 1990).

Survival was assessed by rating plants as normal, weak, or dead 1 month after planting. ANOVA, followed by Bonferroni's test (14), was conducted following angular transformation on the percentage survival of normal plants. Runners and flowers were counted weekly during the first 2 months after planting. The accumulated number of runners and flowers were

subjected to ANOVA, and the main effects were analyzed with Bonferroni's test. The ANOVA for survival and the number of flowers was conducted separately for each year because the year effect was significant ($P = 0.05$). The ANOVA for the number of flowers was also separated for the cultivars because the number of flowers produced from the controls was significantly different among the cultivars. The data from the treatments at 49.4 C were analyzed alone and excluded from the ANOVA for temperatures of 44.4, 46.1, and 47.7 C to achieve homogeneous variances. For plant survival, combined interaction values were used as the error term because these interactions were not significant in preliminary analysis with years as replicates. Contrasts were performed for different times at each temperature for plant survival and flower production (14). The F values for the contrasts were adjusted by the Bonferroni method to control the experiment-wise error rate.

Results

Effect of treatment on nematode mortality and time for crowns to reach a higher temperature: The time required to reach 100% mortality ($P = 0.05$) of *A. fragariae* in water was 15, 5, 4, and 2 minutes at 44.4, 46.1, 47.7, and 49.4 C, respectively (Fig. 1). There was no difference ($P = 0.05$) in the time required to raise the temperature at the crown center from 25 C to a series of higher temperatures ranging from 44.4 to 54.4 C in a hot water bath (data not shown). The linear regression was also not significant ($P = 0.05$).

Effect of treatment of strawberry crowns on subsequent survival, runner, and flower production: The effect of treatment on plant survival was significant ($P = 0.05$) for the factors of temperatures, time, and cultivar (except for the 1988 test) and for the interaction of temperature and time in all 3 years (Table 1). For each year, all temperatures were significantly different from each other in their effects on plant survival ($P = 0.05$). Survival of Selva was lower ($P = 0.05$) than the other cultivars for 2 of the 3 years (1989 and 1990).

The number of runners produced by plants under the conditions of our study was too small to be of value in evaluating the treatment effect. The number of flowers produced by cultivar Pajaro was also too small to be analyzed. The treatment time at each temperature, which did not reduce the number of flowers ($P = 0.05$) and normal plants compared with the untreated, is listed in Table 2. Treatment at 49.4 C for 5 minutes weakened all plants and reduced the number of flowers com-

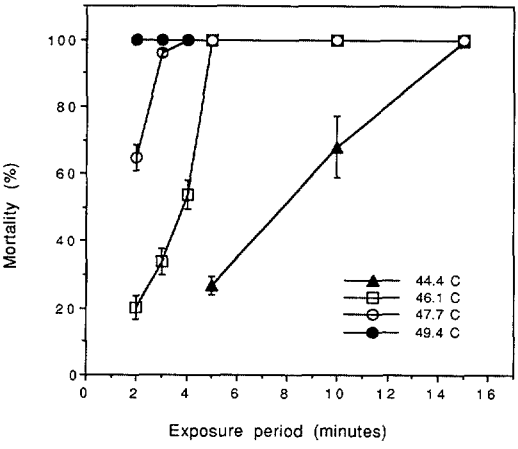

FIG. 1. Mortality (%) of *Aphelenchoides fragariae* in hot water at 44.4, 46.1, 47.7, and 49.4 C. The error bars are standard error of means ($n = 9$).

TABLE 1. Analysis of variance for strawberry plant survival following heat treatment.

Year	Source of variation	df	Mean square	F
1988	Temperature	6	1.41	19.11*
	Time	2	2.43	33.08*
	Temperature × time	12	0.28	3.76*
	Variety	4	0.07	1.00
	Error	80	0.07	
1989	Temperature	6	1.42	32.83*
	Time	2	9.65	222.82*
	Temperature × time	12	0.51	11.78*
	Variety	4	0.42	9.61*
	Error	80	0.04	
1990	Temperature	6	1.53	31.6*
	Time	2	3.10	63.99*
	Temperature × time	12	0.35	7.26*
	Variety	3	0.26	5.34*
	Error	60	0.05	

* Significant at $P = 0.05$.

TABLE 2. Maximum length (minutes) of treatment at various temperatures (C) that did not significantly affect heat-treated strawberry plant growth and flowering compared with untreated plants ($P = 0.05$).

	44.4 C			46.1 C			47.7 C		
Variety	1988	1989	1990	1988	1989	1990	1988	1989	1990
Chandler	10	30	30	20	15	15	10	0	10
Douglas	30	30	—†	10	15	—	10	5	—
Fern	25	30	30	20	20	15	10	10	10
Selva	25	30	20	20	15	15	10	5	10

† — = not tested.

pared with control plants ($P = 0.05$). Although there are some differences in the years and cultivars, the length of treatment, which did not reduce the number of flowers and normal plants compared with controls, was 30, 15, and 10 minutes at 44.4, 46.1, and 47.7 C, respectively (Table 2).

DISCUSSION

We were unable to obtain sufficient quantities of infested strawberry crowns for direct testing of nematode mortality within crowns; however, the time required to provide nematode control in the cultivars tested can be estimated based on the time required to kill extracted nematodes in water and the time for plants to reach the desired treatment temperature in a hot water bath (Table 3). Theoretically, the minimum time required to kill nematodes in crowns would be the time required to kill the extracted nematodes plus the time required to raise the crown temperature to the treatment temperature. Treatment temperature and time should be controlled as accurately as possible, and the volume of water in the bath relative to the volume of material being treated should be large enough that the bath returns to the desired treatment temperature within 1 minute. Based on our experiments, the minimum–maximum time of treatment for management of *A. fragariae* in the cultivars tested is 20–30, 10–15, and 8–10 minutes at 44.4, 46.1, and 47.7 C, respectively. Plant sensitivity at 49.4 C precludes use of this temperature.

Fruit production appears to be more sensitive than plant survival to treatment. Therefore, the minimum time from the range at a given temperature could be used for plants destined for fruit production, and the longer time for propagation material. In general, both the foliar nematodes and strawberry plants are more susceptible to treatment than many other plant-parasitic nematodes and their hosts (2). Shorter exposure times killed our populations of *A. fragariae* compared with populations tested previously (6). The strawberry cultivars tested in our experiments were more sensitive to treatment than pre-

TABLE 3. Time (in minutes) required for management of *Aphelenchoides fragariae* in the strawberry cultivars tested.

	Temperature (C)			
Time	44.4	46.1	47.7	49.4
To kill extracted *A. fragariae*	15	5	4	2
To raise the crown from 25 C to treatment temperature	4	4	4	4
Of theoretical minimum to kill *A. fragariae* in crowns	19	9	8	6
For maximum treatment without affecting plant growth and flowering	30	15	10	0
For minimum—maximum exposure to allow nematode kill but prevent plant damage	20–30	10–15	8–10	0–0

viously tested cultivars (3,7–10,12,13,16). In addition to cultivar, other variables such as crown size and degree of dormancy before treatment could affect survival and vigor. Our results for cultivar Chandler were similar to those reported previously (2).

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