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Evaluation of Hot Water Treatments for Management of *Ditylenchus dipsaci* and Fungi in Daffodil Bulbs[1]

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Abstract: Treatment of daffodil (*Narcissus pseudonarcissus*) bulbs in a 0.37% formaldehyde water solution at 44 C for 240 minutes is a standard practice in California for management of the stem and bulb nematode, *Ditylenchus dipsaci*. Recent concern over the safety of formaldehyde and growers' requests for a shorter treatment time prompted a reevaluation of the procedure. The time (Y, in minutes) required to raise the temperature at the bulb center from 25 to 44 C was related to bulb circumference (X, in cm) and is described by the linear regression $Y = -15 + 3.4X$. The time required for 100% mortality of *D. dipsaci* in vitro without formaldehyde was 150, 60, and 15 minutes at 44, 46, and 48 C, respectively. Hot water treatment (HWT) with 0.37% formaldehyde at 44 C for 150 minutes controlled *D. dipsaci* and did not have a detrimental effect on plant growth and flower production. Shorter formaldehyde-HWT of 90, 45, and 30 minutes at 46, 48, and 50 C, respectively, controlled *D. dipsaci* but suppressed plant growth and flower production. Fungal genera commonly isolated from the bulbs in association with *D. dipsaci* were *Penicillium* sp., *Fusarium oxysporum* f. sp. *narcissi*, and *Mucor plumbeus*, representing 60, 25, and 5%, respectively, of the total fungi isolated. These fungi caused severe necrosis in daffodil bulbs. HWT at 44 C for 240 minutes reduced the number of colonies recovered from bulbs. The effects of formaldehyde, glutaraldehyde, and sodium hypochlorite in reducing the population of fungi within bulbs were variable. Satisfactory control of *D. dipsaci* within bulbs can be achieved with HWT of bulbs at 44 C for 150 minutes with 0.37% formaldehyde or at 44 C for 240 minutes without chemicals.

Key words: daffodil, *Ditylenchus dipsaci*, formaldehyde, glutaraldehyde, hot water treatment, *Narcissus* spp., nematode, sodium hypochlorite, stem and bulb nematode.

Daffodil (*Narcissus pseudonarcissus* L., Amaryllidaceae) is a valuable commercial floral crop in California and is sold as bulbs and cut flowers. Production of daffodils is limited by the stem and bulb nematode, *Ditylenchus dipsaci* (Kühn) Filipjev. Standard nematode management by growers in California is hot water–formaldehyde treatment of bulbs and preplant chemical treatment of soil. The loss of 1,3-dichloropropene (1,3-D) and 1,2-dichloropropane mixture (DD), 1,3-D, aldicarb, and fenamiphos (27,28) leaves only phorate for soil treatment and hot water treatment (HWT) of bulbs with 0.37% formaldehyde at 43.8 C for 240 minutes. This HWT was questioned recently because of uncertainties with registration of formaldehyde, grower perception that the standard hot water–formaldehyde treatment results in flower deformation, and logistical difficulties associated with treating large quantities of bulbs for 240 minutes (28).

HWT with formaldehyde has also been used to control basal rot caused by *Fusarium oxysporum* Schlecht f. sp. *narcissi* Snyder & Hansen (6,10,24). The importance of *F. oxysporum* f. sp. *narcissi* and other fungi located at the center of bulbs in association with *D. dipsaci*, as opposed to rotting of the basal plate by this fungus, has not been addressed adequately. In previous work, HWT without formaldehyde killed the nematode but did not prevent internal rotting of bulbs during storage (21). The purpose of this research was to i) identify the basal hot water temperature and treatment time needed to kill the population of *D. dipsaci* found in California daffodils in relation to bulb size and ii) determine the effects of formaldehyde, sodium hypochlorite, and glutaraldehyde in hot water on *D. dipsaci* and fungi within bulbs as influenced by temperature and duration of treatment.

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The assistance of the daffodil growers of Humboldt County, California, is appreciated.

Materials and Methods

Daffodil bulbs were harvested in June each year for 3 years from a *D. dipsaci*-infested field in Humboldt County, California. The bulbs were stored at 15 C until needed for the experiment, then warmed to 25 C before HWT.

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

Effects of hot water on nematode mortality: Nematodes were extracted from 6-mm^2 slices of 'Fortune' daffodil bulb scales placed in a mist chamber (3) for 12 hours. The nematodes were washed and placed in aerated tap water at 15 C up to 24 hours prior to use in the experiments (7).

The following temperature × time treatments were tested: 42, 44, 46, 48, and 50 C for durations of 15, 30, 60, 90, 120, 150, 180, 210, and 240 minutes; and 48 C for 5 and 10 minutes. Temperature × time combination treatments with less than 100% mortality and the treatments of shortest duration at each temperature that produced 100% mortality were repeated three times. Each run was replicated three times.

The HWT experimental system consisted of 25-ml test tubes containing 9 ml tap water equilibrated to the temperatures of the water bath. One milliliter of nematode suspension containing ca. 50 nematodes (80% juveniles, 10% males, and 10% females) was added to each test tube. The control was a set of test tubes with the nematode suspension held at 25 C. Test tubes were removed from the water bath immediately after completion of the treatment and placed in another water bath to adjust the suspension to 25 C. The tubes were placed in the laboratory (25 C) for 24 to 48 hours, and dead and living nematodes were counted separately. Nematodes responding to a touch with a dissecting needle were considered to be alive (31).

The percentage mortality caused by HWT was calculated as $100 \times [D_t - (N_t \times D_n)]/[N_t - (N_t \times D_n)]$, 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 before analysis of variance (ANOVA) because the variances of means near 0 and 100% were much smaller than those near 50% (19). Preliminary statistical analysis indicated that there were no differences ($P = 0.05$) among the three different runs of this experiment. Therefore, data were pooled, and Duncan's new multiple-range test was performed on the pooled data (1).

Internal bulb temperature × bulb size relationship: Fortune daffodil bulbs with circumferences ranging from 6–20 cm were selected. A temperature probe was inserted into the center of each bulb. The bulbs were placed in water baths at 44, 48, or 50 C until the respective temperatures were achieved. Data were subject to linear regression (1).

Effect of HWT with formaldehyde: Selections of temperatures and times of HWT were based on the previous experiments. Time and temperature treatments with and without 0.37% formaldehyde (made from a formalin solution containing 37% formaldehyde, Mallinckrodt, Paris, KY) were 44 C for 150, 180, 210, and 240 minutes; 46 C for 90, 120, and 150 minutes; 48 C for 60, 90, and 120 minutes; and 50 C for 60 and 90 minutes. The control consisted of untreated bulbs. Each treatment consisted of two replicates (each replicate consisting of one bulb). Each bulb was placed in a small wire basket or nylon bag

and immersed in water for the designated time and temperature. The experiment was conducted twice. Following HWT, bulbs were dried in paper bags in a fume hood with a fan running for 24 hours (25 C) and then stored at 15 C for 14 days, with the assumption that this treatment would provide sufficient time for chemical activity and nematode mortality to occur, before analysis for nematodes and fungi.

The effect of HWT on nematodes was measured by determining the number of bulbs containing living nematodes and the number of living nematodes/gram of bulb tissue. Half of each bulb was weighed, sliced into 6-mm^2 pieces, and placed in a mist chamber for 72 hours (3).

Fungi were isolated from the half of each bulb that was not used for nematode data. Each half bulb was freed of dry scales and surface sterilized for 5 minutes in a 0.525% solution of sodium hypochlorite (NaOCl) (made from 5.25% commercial bleach, Paul Koss Supply Co., Hayward, CA) and rinsed three times with sterile deionized water (DW). The internal scales were cut into 5 × 5 mm pieces. Ten pieces were plated on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) (adjusted pH 4.5–5.0 with 25% lactic acid and incubated) at 25 C with 12-hour light and 12-hour dark periods. The cultures were observed daily for 10–14 days. The fungi were identified to genus based on colony characteristics and fungal morphology. The total number of fungal colonies (FCS) from each plate was pooled for analysis.

Nematode count data was $\log_{10}(X + 1)$ transformed before analysis. Because preliminary statistical analysis indicated that there were no differences ($P = 0.05$) between the two runs of this experiment, data were pooled and Duncan's new multiple-range test was performed on the pooled data (1).

The experiment was also conducted using commercial tanks. Approximately 500 bulbs of Fortune, 'Dutch Master,' 'Golden Harvest,' or a mixture of 'Albert' and Fortune (obtained from a field in which Albert was planted but containing volunteers of Fortune) were treated in a commercial dipping tank containing a 0.37% formaldehyde solution at 44 C for 150 minutes; 46 C for 90, 120, and 150 minutes; 48 C for 45, 75, and 105 minutes; and 50 C for 30, 60, and 90 minutes. The control treatment consisted of untreated bulbs. Following HWT, 10 bulbs (1 bulb represents 1 replicate) from each treatment were randomly selected to assess nematode and fungal survival as described in the previous experiment. The remaining bulbs were planted in a commercial field in Humboldt County, California, during October 1990. Plant growth and flower production were visually rated in February 1991 using a rating scale of 4 = normal, 3 = poor growth or fewer than normal flowers, 2 = very poor growth or many fewer flowers, and 1 = no plants or no flowers.

HWT with sodium hypochlorite (NaOCl): Ten Fortune bulbs (1 bulb = 1 replicate) were exposed to 0, 0.11, 0.26, 0.39, or 0.525% NaOCl solutions at 44 C for 180 and 240 minutes. Untreated bulbs served as controls. The experiment was conducted twice. Nematodes and fungi were assessed as described in experiments previously reported in this paper.

HWT with glutaraldehyde: Fortune bulbs were exposed to 0, 0.025, 0.05, 0.1, or 0.15% glutaraldehyde solutions (made with Ucarcide containing 50% acid glutaraldehyde, Union Carbide Co., Danbury, CT) at 44 C for 240 minutes in the 1989 run of this experiment. The experiment was modified in 1990 using 0, 0.375, 0.5, and 0.75% glutaraldehyde solutions at 44 C for 180 minutes; 0, 0.25, 0.375, 0.5, and 0.75% glutaraldehyde at 44 C for 240 minutes; and 0.37% formaldehyde at 44 C for 240 minutes as a standard. In 1991, 0 and 0.75% glutaraldehyde solutions were used at 44 C for 240 minutes. Untreated bulbs served as controls. Nematodes (except in the 1991 experiment) and fungi were assayed as described earlier in this paper.

Pathogenicity of fungi to daffodils: The three most frequently isolated fungi, *Penicillium* sp., *F. oxysporum* f. sp. *narcissi,* and

Mucor plumbeus Bonord (=*M. spinosus* Tiegh), and a less frequently isolated fungus (Unidentified Isolate No. 912) were tested in May 1991 for their pathogenicity to daffodil. The identification of *F. oxysporum* f. sp. *narcissi* was confirmed by S. N. Smith, and the identification of *M. plumbeus* was confirmed by E. E. Butler, both from the Department of Plant Pathology, University of California, Davis.

The dried outer layer of Fortune bulb scales was removed from 30 healthy bulbs. The bulbs were washed with tap water, dried with paper towels, surface sterilized in 0.525% NaOCl for 5 minutes, and rinsed three times with sterile DW. The top of each bulb was cut with a sterile blade and removed. Bulbs were placed individually in sterile petri dishes (60 × 15 mm) containing 1 ml of sterile DW. The petri dishes with bulbs were enclosed in polystyrene containers on layers of wet paper towels. The cut surface of each bulb was inoculated with a 5-mm-d agar disc cut from the outer margin of a 7-day-old-culture (produced from a single spore of each fungus) on PDA. Controls were cut bulbs with and without a sterile agar disc. The six treatments (four fungi and two controls) were replicated five times. A replicate consisted of one bulb per treatment placed in an individual polystyrene box, which was covered, sealed, and incubated on a laboratory bench at 25 C with 12-hour light and 12-hour dark periods. After 4 days of incubation, the depth and width of brown necrotic tissue was measured for each bulb. Fungi were re-isolated from the bulbs and identified to satisfy Koch's postulates.

Results

Effects of hot water on nematode mortality: Exposure time for mortality of *D. dipsaci* in hot water was inversely related to temperature (Fig. 1). The maximum mortality at 42 C was 32%. The time required to achieve 100% mortality was 150, 60, 15, and 15 minutes at 44, 46, 48, and 50 C, respectively (data for 50 C are not shown in Figure 1, and times shorter than 15 minutes were not tested).

Internal bulb temperature × bulb size relationship: Linear regressions for the time required to raise the temperature from 25 C to 44, 48, or 50 C at the center of bulbs with different circumferences, respectively, were $Y = -15 + 3.4X$, $R^2 = 0.82$, $P < 0.01$; $Y = -10 + 2.7X$, $R^2 = 0.81$, $P < 0.01$; and $Y = -14 + 3.3X$, $R^2 = 0.71$, $P < 0.01$, where Y = time (minutes) and X = bulb circumference (cm). The length of time required to reach the three experimental temperatures significantly increased as bulb circumference increased. The regression slopes and intercepts among the three temperatures were not different ($P = 0.05$). Thus, we combined all data from the three temperatures in a single linear regression, described by $Y = -11.4 + 3X$ ($R^2 = 0.78$ and $P < 0.01$) (Fig. 2).

Effect of HWT with formaldehyde: All control bulbs contained *D. dipsaci* with an average of 300 nematodes/g bulb (Table 1). The number of bulbs with surviving nematodes and numbers per gram of bulb were reduced by HWT. Addition of formaldehyde was beneficial only at 150- and 180-minute exposures at 44 C. The linear regression of mean nematode counts over time for HWT without formaldehyde at 44 C for 150 to 240 minutes showed a

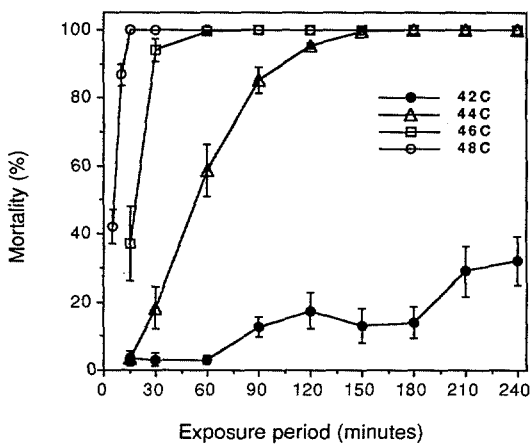

FIG. 1. Mortality (%) of *Ditylenchus dipsaci* after hot water treatments in vitro at 42, 44, 46, and 48 C. The error bars are standard error of means ($n = 9$).

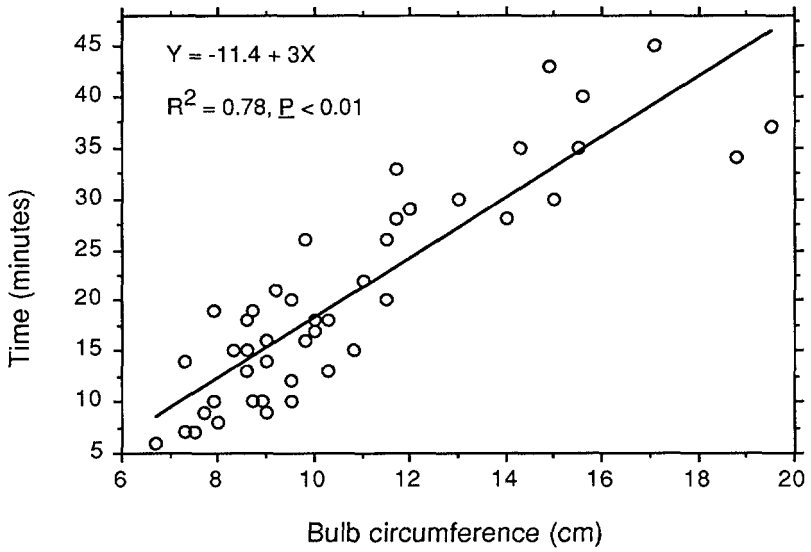

FIG. 2. Time required to raise the temperature at the center of daffodil bulbs from 25 C to 44, 48, or 50 C in a hot water bath.

trend for increased nematode control with increasing time ($Y = 20.25 - 0.085X$, $R^2 = 0.98$, $P < 0.01$, where Y = number of nematodes and X = time).

Compared to untreated bulbs, HWT, with or without formaldehyde, did not affect the number of fungal colonies recovered from treated bulbs (Table 1). However, when data from all exposure periods at a single temperature were grouped, the effect of formaldehyde in decreasing ($P = 0.05$) the number of FCS at 44, 46, 48, and 50 C could be demonstrated. Individual mean comparison of treatments with and without formaldehyde showed that the presence of formaldehyde reduced (P =

TABLE 1. Effect of hot water treatment with (+) or without (−) 0.37% formaldehyde on *Ditylenchus dipsaci* and fungi within Fortune daffodil bulbs.

		Bulbs with *D. dipsaci*			*D. dipsaci*/g bulb			FCS/10 pieces of bulb†		
Temperature (C)	Time (min)	+	−		+	−		+	−	
44	150	1 b	2 abc	NS	0.25 b	7.50 b	S	5.5 a	9.3 ab	NS
	180	0 b	3 bc	S	0 b	5.13 b	S	5.4 a	14.3 a	S
	210	0 b	2 abc	NS	0 b	1.75 b	NS	4.5 a	12.5 ab	S
	240	0 b	2 abc	NS	0 b	0.10 b	NS	4.5 a	7.3 bc	NS
46	90	0 b	2 abc	NS	0 b	1.15 b	NS	3.0 a	12.8 ab	S
	120	2 ab	0 c	NS	0.05 b	0 b	NS	7.3 a	10.8 ab	NS
	150	0 b	0 c	NS	0 b	0 b	NS	4.8 a	8.8 ab	NS
48	60	0 b	1 bc	NS	0 b	0.60 b	NS	7.3 a	12.0 ab	NS
	90	2 ab	2 abc	NS	0.3 b	2.08 b	NS	6.8 a	13.3 ab	S
	120	0 b	0 c	NS	0 b	0 b	NS	3.3 a	5.8 c	NS
50	60	1 b	0 c	NS	0.03 b	0 b	NS	6.8 a	12.0 ab	NS
	90	0 b	1 bc	NS	0 b	0.03 b	NS	4.8 a	10.3 ab	S
Control (no formaldehyde)		4 a	4 a		300 a	300 a		8.5 a	8.5 abc	

Number of nematodes and fungal colonies are means of two runs of two replicates per run (1 bulb/replicate). Nematode counts were transformed by $\log10(X + 1)$ before statistical analyses. Means within a column followed by the same letter are not different ($P = 0.05$) according to Duncan's new multiple-range test. S and NS refer to significant and nonsignificant differences, respectively, between adjacent columns.

† Fungal colonies (FCS) consisted primarily of *Penicillium* sp., *Fusarium oxysporum* f. sp. *narcissi*, and *Mucor plumbeus*.

0.05) FCS at 44 C exposed for 180 and 210 minutes, and at 46, 48, and 50 C for 90 minutes.

In the experiment utilizing commercial tanks, in which all HWT contained formaldehyde, all of the untreated Fortune and Golden Harvest bulbs and 30% of the Dutch Master and the mixture of Fortune and Albert bulbs contained *D. dipsaci* (Table 2; only the data from Fortune cultivar are shown because data for other cultivars were similar but more variable). All treatments reduced the number of bulbs with surviving nematodes and reduced the number of *D. dipsaci* per gram of bulb (P = 0.05). The number of FCS in bulbs exposed to 44 C for 150 minutes, 46 C for 90 and 150 minutes, 48 C for 105 minutes, and 50 C for 90 minutes was lower (P = 0.05) than in untreated bulbs.

The only treatment with acceptable field performance with respect to plant growth, flower quantity, and flower quality was that in which bulbs were HWT at 44 C for 150 minutes (Table 2). Treatments at 46 C for 90, 120, and 150 minutes resulted in marginal plant performance. Temperatures above 46 C resulted in unacceptable plant growth and flower production. No flowers were produced by untreated bulbs.

HWT with sodium hypochlorite: All untreated bulbs contained nematodes, with an average of 862 *D. dipsaci* per gram of bulb (Table 3). HWT reduced (P = 0.05) the number of bulbs with surviving nematodes and the number of nematodes per gram of bulb, whether NaOCl was present or absent (Table 3). The number of FCS isolated from the 180-minute exposure to 0 and 0.11% NaOCl at 44 C increased (P = 0.05) compared to the non-HWT. Exposures to higher concentrations of NaOCl for 180 minutes did not affect the number of FCS. HWT at 44 C for 240 minutes reduced the number of FCS, regardless of the concentration of NaOCl.

HWT with glutaraldehyde: HWT with and without glutaraldehyde at 44 C for 180 or 240 min reduced (P = 0.05) the number of bulbs with surviving nematodes and the number of nematodes per gram of bulb (Table 3). The fungal data from 180-minute exposures were quite variable (Table 3). HWT alone increased the number of FCS, and their recovery was decreased from HWT with 0.375 and 0.5% glutaraldehyde. HWT at 44 C for 240 minutes reduced (P = 0.05) the number of FCS compared to untreated bulbs. Addition of 0.75% glutaraldehyde in the HWT was the

TABLE 2. Effect of commercial tank hot water treatment with 0.37% formaldehyde on *Ditylenchus dipsaci* and fungi within Fortune daffodil bulbs and on plant growth and flower production.

Temperature (c)	Time (min)	Number of bulbs with *D. dipsaci*†	Number *D. dipsaci*/g bulb‡	FCS/10 pieces of bulb‡	Plant growth§	Flower§	
						Quantity	Quality
44	150	2 bc	0.05 b	1.2 d	4	4	4
46	90	1 bc	1.85 b	0.5 d	4	4	3
	120	1 bc	4.40 b	5.6 ab	4	2	2
	150	1 bc	0.10 b	2.3 cd	4	4	3
48	45	1 bc	0.60 b	4.6 bc	2	4	2
	75	0 c	0 b	2.7 bcd	3	3	2
	105	1 bc	0.10 b	2.4 cd	2	2	2
50	30	1 bc	0.90 b	2.7 bcd	2	2	2
	60	5 b	6.06 b	8.1 a	2	2	2
	90	2 bc	0.43 b	2.1 cd	2	2	2
Control (no formaldehyde)		10 a	52.50 a	5.7 ab	2	1	1

† χ^2 Goodness of fit testing was conducted for these data, followed by a Z-test for multiple comparisons. Numbers within a column followed by the same letter are not significantly different at P = 0.05 adjusted by the Bonferroni method.

‡ Number of nematodes and fungal colonies (FCS) are means of 10 replicates (1 bulb/replicate). Nematode numbers were transformed by $\log10(X + 1)$ before statistical analyses. Means within a column followed by the same letter are not significantly different at P = 0.05 according to Duncan's new multiple-range test.

§ The numbers in columns are visual ratings of plant growth, flower quantity and quality: 4 = normal; 3 = poor growth or fewer than normal flowers; 2 = very poor growth or many fewer than normal flowers; 1 = no plants or no flowers.

TABLE 3. Effect of hot water treatment with sodium hypochlorite (NaOCl) or glutaraldehyde (Glutar) at 44 C on *Ditylenchus dipsaci* and fungal survival within Fortune daffodil bulbs.

Time (min)	NaOCl (%)	Glutar (%)	Number of bulbs with *D. dipsaci*†		Number of *D. dipsaci*/g bulb‡		CFU/10 pieces of bulb‡	
			NaOCl	Glutar	NaOCl	Glutar	NaOCl	Glutar
0	0	0	10 a	10 a	862 a	862 a	7.8 b	7.8 b
180	0	0	2 b	2 b	<1 b	<1 b	11.0 a	11.0 a
	0.11	0.375	2 b	2 b	<1 b	<1 b	10.9 a	2.6 cd
	0.26	0.5	2 b	2 b	<1 b	1 b	6.5 bc	2.0 cd
	0.39	0.75	0 b	3 b	0 b	1 b	6.0 bcd	5.4 bc
	0.525		4 b		3 b		6.8 bc	
240	0	0	2 b	2 b	<1 b	<1 b	4.1 cd	4.1 c
	0.11	0.25	2 b	0 b	<1 b	0 b	3.8 cd	3.0 cd
	0.26	0.375	0 b	0 b	0 b	0 b	3.0 d	2.3 cd
	0.39	0.5	0 b	1 b	0 b	<1 b	3.0 d	4.5 bc
	0.525	0.75	0 b	1 b	0 b	<1 b	3.6 cd	0.2 d
	0.37§		1 b		<1 b		1.8 cd	

† χ^2 goodness-of-fit testing was conducted for these data, followed by the Z-test for multiple comparisons. Numbers within a column followed by the same letter are not significantly different at $P = 0.05$ adjusted by the Bonferroni method.

‡ Number of nematodes and fungal colonies (FCS) are means of 10 replicates (1 bulb/replicate). Nematode numbers were transformed by $\log10(X + 1)$ before statistical analyses. Means within a column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's new multiple-range test.

§ Formaldehyde was used in this treatment.

only concentration that provided greater reduction ($P = 0.05$) of FCS than the HWT alone.

Untreated stored daffodil bulbs were infected with *Penicillium* spp., *Fusarium* spp., *F. oxysporum* f. sp. *narcissi*, *Mucor* spp., *M. plumbeus*, *Alternaria* sp., *Botrytis* sp., *Geotrichum* sp., *Rhizopus* sp., *Stemphylium* sp., and *Trichoderma* sp., as well as some fungi that were not identified. *Penicillium* sp., *F. oxysporum* f. sp. *narcissi*, and *M. plumbeus* were the most frequently isolated fungi, with frequencies of 60, 25, and 5% of total FCS, respectively. Numbers of *D. dipsaci* were positively related to frequency of isolation of FCS ($Y = 0.075X + 0.693$, $R^2 = 0.358$, $P = 0.05$).

Pathogenicity of fungi to daffodils: No symptoms were observed from the two sets of control bulbs. All bulbs inoculated with *Penicillium* sp. developed necrotic tissue averaging 13 mm in depth and 4.5 mm in width in 4 days. *Fusarium oxysporum* caused lesions 3.5 mm deep and 1.12 mm wide. Damaged tissue size for *M. plumbeus* was 2.8 mm deep and 0.5 mm wide. An unidentified fungus caused only slight tissue damage with lesions 1 mm deep and 0.08 mm wide. Koch's postulates were satisfied by re-isolating the fungi.

DISCUSSION

The minimum exposure time required to kill *D. dipsaci* within bulbs is equal to the time required to kill *D. dipsaci* in vitro plus the time required to raise the bulb center to the temperature of the water. Because many nematodes are found in the center of bulbs (30), the durations of HWT must consider bulb size and the time required to heat it to the desired temperature. The size of these bulbs varies among cultivars. In our experiments, average bulb circumferences of Albert, Fortune, Dutch Master, and Golden Harvest were 9, 10, 13 and 13 cm, respectively, and therefore would require 16, 19, 29, and 29 minutes, respectively, to raise the temperature from 25 C to 44 C in a water bath based on the model $Y = -15 + 3.4X$. Therefore, the length of HWT should be adjusted for size of bulbs. If the size of commercially harvested bulbs is not uniform, they should be sorted by size.

Nematode mortality in HWT for the population of *D. dipsaci* found in Humboldt County, California, is similar to that obtained for this nematode in other countries (7,22). Saigusa and Yoshihara (22) found that 12 hours at 42.5 C and 4 min-

utes 11 seconds at 50 C are required to reach 100% mortality. However, neither of these times is practical for commercial scale HWT.

Sodium hypochlorite solution reduces the germination of conidia of *Penicillium expansum* and *Mucor* spp. (4,23). Some researchers indicate that NaOCl does not penetrate plant materials very well (25). However, because of its widespread use and perceived safety, we felt that it was worth testing as a potential replacement for formaldehyde. Glutaraldehyde is widely used as a biocide in cosmetics (2). Both alkaline and acid glutaraldehyde have been tested for control of various bacteria and fungi (2,26). Linfield (18) reported that 0.25% to 1.5% alkaline glutaraldehyde in HWT at 44.4 C for 160 minutes killed 100% of the chlamydospores of *F. oxysporum* f. sp. *narcissi* in vitro; and exposures to 0.1% and 0.5% glutaraldehyde in HWT at 44.4 C for 180 minutes did not decrease flower quality or quantity of Golden Harvest, 'Carlton,' 'Ice Follies,' or 'St. Keverne.' In our experiments, suppression of fungi was observed with 0.26% NaOCl and 0.375% glutaraldehyde in HWT at 44 C for 180 minutes compared with HWT alone. There was no additional benefit from either chemical additive for fungal suppression or nematode control over HWT alone at 44 C for 240 minutes because HWT alone suppressed fungi and controlled nematodes. A treatment time of 150 minutes at 44 C with NaOCl or with glutaraldehyde should be further tested for control of *D. dipsaci* and fungi, and for effects on following plant growth and flower production.

In our experiments and those of others (5,15,29), some inconsistencies were seen in nematode survival in the various experiments with increasing chemical concentration and exposure time. We were not able to eradicate all the nematodes within the bulbs by hot water, chemical treatments, or a combination of both, especially within heavily infested bulbs. Many of these heavily infested bulbs are soft to the touch and would be culled during commercial harvest, although some are firm and appear normal from the outside. Some of the normal looking but heavily infested bulbs were used in the experiment with 0.525% NaOCl and could have caused the abnormal results in this experiment. The inconsistencies encountered in the experiments could also be due to the nonuniform or skewed distribution of nematodes within bulbs, which has been well documented by Hesling (12,14). Attempts to produce uniformly infested bulbs have not been successful (13).

These are reports of the association of *D. dipsaci* with fungi or bacteria on several crops (8,11,20). *M. plumbeus* has been isolated from pecans (16) and maize (9) but has not been reported from daffodil bulbs. The importance of *Fusarium* basal rot in daffodil and its control has been extensively studied (10,18,24). Other fungi associated with rotting bulbs infested by *D. dipsaci* have been mentioned in a disease survey only (17). *F. oxysporum* f. sp. *narcissi*, *Penicillium* sp., and *M. plumbeus* are important internal storage pathogens of daffodil bulbs. The role of such pathogens should be considered in future experiments and in the management of *D. dipsaci* and fungi within daffodil bulbs.

In summary, the time of HWT should vary according to bulb size. Bulbs should be sorted by size and soft bulbs removed. Satisfactory control of *D. dipsaci* within bulbs can be achieved with 0.37% formaldehyde HWT at 44 C for 150 min, or with HWT alone at 44 C for 240 min. A preplant or at planting nematicide treatment would be warranted if nematodes are present in the soil. Postplant soil or foliar nematicide applications may be warranted if bulbs are to remain in the ground for more than one season.

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