Disinfection Alternatives for Control of *Ditylenchus dipsaci* **in Garlic Seed Cloves 1**

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Abstract: Hot-water dips with and without the additives abamectin and sodium hypochlorite were evaluated for control of *Ditylenchus dipsaci* infection of garlic seed cloves. All treatments were compared to hot water-formalin clove dip disinfection and to nontreated infected controls for garlic emergence, midseason infection, bulb damage, and yield at harvest in field plots in 12 experiments. Hot-water treatments without additives only partially controlled *D. dipsaci* when a warming presoak dip (38 C) of 30, 45, or 60 minutes' duration was followed by a hot-water dip (49 C) of 15-30 minutes' duration. Exposure to 49 C for 30 minutes caused slight retardation of garlic emergence, although normal stand was established. Abamectin at 10-20 ppm as the 20-minute hot dip (49 C) or as a 20-minute cool dip (18 C) following a 20-minute hot-water dip and sodium hypochlorite at 1.052-1.313% aqueous solution as the 20-minute hot dip were highly effective in controlling D. *dipsaci* and were noninjurious to garlic seed cloves. None of these treatments was as effective as a hot water-formalin dip and were noneradicative, but showed high efficacy on heavily infected seed cloves relative to nontreated controls. Abamectin was most effective as a cool dip. These abamectin cool-dip (following hot-water dip) and sodium hypochlorite hot-dip treatments can be considered as effective alternatives to replace formalin as a dip additive for control of clove-borne *D. dipsaci.* Sodium hypochlorite was less effective as the cool dip, and at concentrations of 1.75-2.63% was phytotoxic to garlic.

Key words: abamectin, *Allium sativum, Ditylenchus dipsaci,* formaldehyde, garlic, hot-water dip, seedborne infection, sodium hypochlorite, stem nematode.

The stem and bulb nematode, *Ditylenchus dipsaci* (Kfihn) Filipjev, is a serious pest of commercial garlic *(AUium sativum* L.) in production areas worldwide. *Ditylenchus dipsaci* infections can arise from planting in nematode-infested soil or more commonly from planting infected garlic seed cloves. In California, where most of the garlic in the United States is produced, a rotation of 4 years of nonhost crops between garlic plantings effectively manages soil infestations of *D. dipsaci.* Crop injury from seed clove infection has been prevented by disinfection of cloves with a hot waterformalin dip treatment $(9,10,13)$, combined with efforts to produce nematodefree planting stock. Phenamiphos applied in planting furrows also is effective for control of seedborne infection (8,13).

Hot water-formalin dip treatments were first developed for nematode and fungus disinfection of narcissus bulbs (1,2,7,19) and subsequently have been used effectively to control *D. dipsaci* in other bulbous plants (1,3,12). Lear and Johnson (9,10) refined these hot water-formalin treatments for disinfection of the much smaller garlic cloves. The standard hot waterformalin dip treatment for commercial garlic seed cloves is a modified version of that developed by Lear and Johnson. Seed clove lots are immersed in a warming dip of water for 30 minutes at 38 C followed by immersion in 0.74% aqueous formaldehyde for 20 minutes at 49 C, then 10 minutes in 0.06% benomyl at 18 C and air dried. The formaldehyde solution has been used also in the warming stage but is not necessary, and concentrations of 0.2- 1.0% aqueous formaldehyde have been used effectively for garlic and other bulbous plants (10,12,13). Benomyl in the cooling stage is used for surface sterilization to minimize fungal contamination (8).

The use of formaldehyde in the dip treatment is now prohibited due to worker safety concerns. Previous reports on disinfection efficacy of hot-water treatment without addition of formaldehyde indicated only partial control at temperatureexposure time combinations that were

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nondamaging to garlic seed cloves (9,10). In vitro studies on *D. dipsaci* mortality rates and thresholds with different temperature and exposure time combinations in aqueous solutions revealed much more rapid mortality at 48 C than at lower temperatures (6,12,20). The time required for 100% mortality was 150, 60, and 15 minutes at 44, 46, and 48 C, respectively (12). Both preconditioning and posttreatment storage temperatures, but not the rates of heating and cooling, were shown to influence resistance of *D. dipsaci* to high temperature (6,19).

Our objectives were to investigate the disinfection potential of hot-water dip treatments within the thermal tolerance limits of garlic cloves, both i) without chemical additives and ii) with abamectin and sodium hypochlorite as dip additives. Abamectin and sodium hypochlorite were the most promising compounds selected from preliminary screening of numerous alternative additives with lower perceived health and environment risks than formaldehyde. A preliminary report of this work has been given (14).

MATERIALS AND METHODS

Allium sativum cv. California Late naturally infected with *D. dipsaci* was used in all experiments. Cloves were selected from bulbs harvested from field plantings that showed light to moderate disease symptoms the previous season.

During the week before treatment and planting, bulbs were mechanically separated into cloves. The complete batch of cloves was mixed thoroughly, and approximately 15-kg sub-lots were placed in coarse nylon-mesh field sacks to facilitate immersion in treatment baths. Treatment baths were cylindrical with a capacity of 208 liters, and were fitted with a circulation pump and a thermostatically controlled heating element that maintained treatment temperature within ± 0.3 C. Airdrying was done within 24 hours of treatment by placing the sacks of cloves on forced-air driers.

In each experiment disinfection treatments were compared with a nontreated control that was dipped in 0.06% benomyl at 18 C for 10 minutes and air-dried, and with standard hot water-formalin dip treatment (30 minutes in water at 38 \overline{C} , then 20 minutes in 0.74% aqueous formaldehyde at 49 C, followed by 10 minutes in 0.06% benomyl at 18 C and air-dried). Experimental disinfection treatments were modifications of the standard hot-water dip treatment by omitting or substituting different concentrations of chemical additives, varying times of temperature exposure, or placing additives in the hot or cool stages of dip treatment. Sodium hypochlorite dip solutions were made from dilutions in water of commercial bleach (Clorox 5.25% [v/v] aqueous sodium hypochlorite). Abamectin dip solutions were made from dilutions in water of 87.4 g a.i./liter EC of abamectin (Merck and Co., Rahway, NI). Treatment details are given in the data tables.

Subsamples of 100 cloves from each sublot were taken immediately before treatment and after treatment and drying, and diced. *Ditylenchus dipsaci* was extracted from diced cloves in modified Baermann funnels in a mist chamber for 5 days. Extracted nematodes were counted.

Experiments were conducted on field sites not infested with *D. dipsaci,* at Five Points (sandy clay loam soil, experiments 1, 3, 5-12) and Parlier (sandy loam soil, experiments 2, 4) in the San Joaquin Valley, California. Within an experiment each treatment was replicated four (experiments $1-6$) or five (experiments $7-12$) times in a randomized complete block design. Each replicate consisted of two 3-m rows planted 30 cm apart on a single 1-mwide bed. A 3-m-long buffer of nematodefree garlic or fallow bed separated replicates linearly along a bed, and experimental beds were buffered on each side with two beds of nematode-free garlic.

Garlic culture followed the standard commercial procedures for California Late (18). Planting was done between mid-October and mid-November, and harvest was carried out in late July to early August. Garlic emergence data were collected on three dates by counting total numbers of plants emerged following hand-planting of 10 cloves/30 cm of row (200 cloves/ replicate plot). Infection estimates were made by visual observation of plants with typical above-ground infection symptoms (stem bloating, leaf yellowing, and wilting).

Approximately 3 weeks before harvest, bulbs were mechanically undercut to separate roots and facilitate drying. At harvest, alt bulbs were dug and the tops cut off by hand. Bulbs were separated into infectiondamage categories of "none" = no symptoms, "slight/moderate" = discoloration but acceptable for commercial processing, and "severe" = not commercially acceptable. Bulbs in each category were counted and weighed. Data were analyzed with the General Linear Models Procedure (SAS Institute, Cary, NC) for ANOVA F test, followed by Duncan's multiple-range test for variables with significantly different (P) $= 0.05$ means.

RESULTS

Hot-water treatments: Hot-dip (49 C) treatment times of 15, 20, 25, and 30 minutes in water without formaldehyde following a 30-minute warming dip did not affect final stand establishment based on plant emergence in four experiments (Table 1). However, in experiment 4 both 25and 30-minute hot-dips retarded early plant emergence, even though a normal final stand developed (Table 1). The hotwater dip times of 15-30 minutes without additives in most cases suppressed D. *dipsaci* infection assessed in midseason, compared with the nontreated controls in the four experiments (Table 2). However, infection was still significantly higher than in the hot water-formalin dip treatment, and no trends of improved control were associated with extension of the hot-dip duration (Table 2). The mean number of bulbs in the "none" and "slight/moderate" harvestable categories demonstrated that only partial control of *D. dipsaci* was achieved by hot water without additive treatments in experiments $1-4$ (Table 2).

Extension of the warming dip from the standard 30 minutes to 45 and 60 minutes followed by 20- or 30-minute hot dip did not significantly improve suppression of D. *dipsaci* infection and damage by hot-water dipping without additives (Table 3). In both experiments 5 and *6, D. dipsaci* initial infection levels were light and apparently unevenly distributed, but they were still adequate to assess treatment effects. Partial suppression of *D. dipsaci* infection was achieved by most hot-water treatments without additives. However, *D. dipsaci* control was significantly less than hot waterformalin dip treatment in one or more comparisons based on the number of infected plants in midseason or severely

TABLE 1, Effects of hot-water dip time and hot water-formalin dip treatments on garlic emergence $(experiments 1-4).$

Treatment ^a	Emergence %				
	Expt. 1 Day $126b$	Expt, 2 Day 126	Expt. 3 Day 132	Expt. 4	
				Day 55	Day 132
Hot water-formalin dip	93.5 b	98.0a	92.9ab	77.9 a	93.7a
$30/15/10$ water dip	95.9 ab	97.5a	93.4 ab	76.8 a	91.3 a
$30/20/10$ water dip	93.9 b	97.9a	93.2 ab	77.9 a	93.7a
$30/25/10$ water dip	95.9 ab	96.3a	90.4 _b	67.2 b	92.5a
$30/30/10$ water dip	94.5 b	96.3a	95.0a	56.7c	91.9 a
Control	97.2a	91.8 b	95.2a	75.4a	92.9a

a Treatment times (minutes) in warming dip (38 C)/hot dip (49 C)/cool dip (0.06% benomyl at 18 C) stages; all treatments air-dried after cool dip.

^b Days after planting.

TABLE 2. Effects of hot-water dip time and hot water-formalin dip treatments on *Ditylenchus dipsaci* midseason garlic infection and numbers of harvestable bulbs (experiments 1-4).

a Treatment times (minutes) in warming dip (38 C)/hot dip (49 C)/cool dip (0.06% benomyl at 18 C) stages; all treatments air-dried after cool dip.

b Mean initial *D. dipsacilclove* before treatment 0.1-217 (expt. 1); 0.1-59 (expt. 2); 31-111 (expt. 3); 0.3-31 (expt. 4).

Values in each column followed by a common letter are not significantly different ($P = 0.05$).

damaged bulbs at harvest (Table 3). None of the treatments significantly affected plant emergence and stand establishment. The overall failure of hot-water dip treatments without additives to suppress D. *dipsaci* infection was confirmed in experiment 7 for the 30/20/10 and 60/20/10 water-dip treatment time regimes (Table 4).

Hot-water-additive treatments: Abamectin at concentrations of 10-50 ppm used as the hot-dip (49 C) stage or as the cool-dip (18 C) stage was effective in significantly reducing *D. dipsaci* infection in five experiments (Tables 4–7). Although abamectin treatments overall were not as effective as hot water-formalin dip, good levels of control were manifested in low midseason infection, reduced bulb damage at harvest, and increased yield of bulbs. Control of D. *dipsaci* by abamectin concentrations of 10

and 20 ppm was greater in the cool-dip (18 C) stage (Tables 5,6). Effectiveness of abamectin at both concentrations as the cool dip was increased when cool-dip duration was extended from 10 to 20 minutes (Tables 5-7); 10 ppm of abamectin for 10 minutes was the least effective of these concentration \times duration cool-dip combinations (Table 5). In experiment 9, 20 ppm of abamectin as a 20-minute cool dip without the preceding 20-minute hot-water dip (49 C) stage did not provide effective *D. dipsaci* control, compared with the same cool dip preceded by the standard hot-water dip (Table 6).

Abamectin treatments did not significantly affect plant emergence compared with hot water-formalin dip in experiments 8-11 (Tables 5,6; data not shown for experiments 10 and 11). In experiment

Treatment times (minutes) in warming dip (38 C)/hot dip (49 C)/cool dip (0.06% benomyl at 18 C) stages; all treatments air-dried after cool dip.
^b Mean initial *D. dipsaci/c*love before treatment 81–405 (expt. 5), 23–230 (expt. 6).

TABLE 4. Effects of hot-water dip time and hot-water dip additive treatments on *Ditylenchus dipsaci* midseason garlic infection, garlic emergence (161 days after planting), and weight of harvestable bulbs (experiment 7).

^a Treatment times (minutes) and additives in warming dip (38 C)/hot dip (49 C)/cool dip (0.06% benomyl at 18 C) stages; all treatments air-dried after cool dip.

b Mean initial *D. dipsaci/clove* before treatment 27-110.

Values in each column followed by a common letter are not significantly different ($P = 0.05$).

7, conducted during an abnormally cold winter growing season and with heavily infected seed clove lots, poor plant emergence occurred in all treatments (e.g., only 75.5% for hot-water formalin dip), and abamectin significantly suppressed emergence, most notably at the 50-ppm concentration (Table 4). In an additional experiment (experiment 12, data not shown) with healthy, uninfected commercial seed cloves, 20 ppm of abamectin as a 20 minute hot-dip or cool-dip treatment was not phytotoxic.

Aqueous sodium hypochlorite (NaOC1)

concentrations of 1.052% to 2.63% as the 20-minute hot-dip (49 C) stage was effective in significantly controlling *D. dipsaci* infection in four experiments (Tables 4,6,7). These NaOC1 hot-dip treatments were similar to the best abamectin treatments, indicated by low midseason infection and reduced nematode damage to bulbs at harvest. Both 1.052% and 1.313% NaOCl as the 20-minute cool-dip stage were also effective in controlling *D. dipsaci* infection (Table 7). However, slightly higher total numbers of infected plants and "slight-moderate" damaged bulbs at

TABLE 5. Effects of dip time and additive placement and concentration treatments on *Ditylenchus dipsaci* midseason garlic infection, garlic emergence (147 days after planting), and weight of harvestable bulbs (experiment 8).

^a Treatment times (minutes) and additives in warming dip (38 C)/hot dip (49 C)/cool dip (0.06% benomyl at 18 C) stages; all treatments air-dried after cool dip.

b Mean initial *D. dipsaci/clove* before treatment 633-1,283.

TABLE 6. Effects of dip time and additive placement and concentration treatments on *Ditylenchus dipsaci* midseason garlic infection, garlic emergence (147 days after planting), and weight of harvestable bulbs (experiment 9).

Treatment times (minutes) and additives in warming dip (38 C)/hot dip (49 C)/cool dip (0.06% benomyl at 18 C) stages; all treatments air-dried after cool dip.

b Mean initial *D. dipsaci/clove* before treatment 550-1,228.

Values in each column followed by a common letter are not significantly different ($P = 0.05$).

harvest suggest that NaOCl is less effective when used in the cool dip than in the hotdip stage.

Solutions of 1.75% and 2.63% NaOC1 as the 20-minute hot-dip stage appeared to be phytotoxic to seed cloves weakened by heavy *D. dipsaci* infection, causing significant suppression of plant emergence and low overall bulb yield (Tables 4,6). However, 1.75% NaOCI as a 20-minute hot dip was not phytotoxic to healthy, uninfected commercial seed cloves (experiment 12, data not shown). Solutions of 1.052% (three experiments) and 1.313% (one experiment) NaOC1 as 20-minute hot-dip or

cool-dip stages did not suppress plant emergence or overall bulb yield (Tables 6,7).

DISCUSSION

Hot-water dipping of garlic seed doves without the use of dip additives to control clove-borne *D. dipsaci* would be highly desirable. However, the upper limits of thermal tolerance of garlic seed cloves and of the life stages of *D. dipsaci* are similar enough to prevent complete disinfection of cloves by hot-water dipping without injuring the garlic. Based on our results here

TABLE 7. Effects of dip time and additive placement and concentration treatments on *Ditylenchus dipsaci* midseason garlic infection (experiments 10 and 11) and weight of harvestable bulbs (experiment 10).

a Treatment times (minutes) and additives in warming dip (38 C)/hot dip (49 C)/cool dip (0.06% benomyl at 18 C) stages; all treatments air-dried after cool dip.

b Mean initial D. *dipsaci/clove* before treatment 4-106 (expt. 10); 42-84 (expt. 11).

c Not tested.

and the work of Lear and Johnson (9,10), garlic is readily injured by a few minutes' exposure to temperatures above 49 C. Therefore, we attempted to modify hotwater dipping without additives to maximize *D. dipsaci* disinfection without injuring the seed cloves. Extension of the hotdip (49 C) duration to 30 minutes and extension of the presoak warming-dip duration to 60 minutes failed to consistently improve effectiveness of the hot-water dip treatment. At 30 minutes of hot-dip exposure, garlic emergence was retarded, and although a full, final plant stand was established, slow emergence suggests that this duration at 49 C is at the injury limit for cloves. Extending the presoak warmingdip duration did not lower disinfection efficacy, as might be expected if it had a conditioning effect on *D. dipsaci* to resist heat effects. However, reports on preconditioning-induced resistance have involved exposure of nematodes in vitro or in infected narcissus bulbs for several days or weeks (6,19). The presoak warming dip is included to gradually raise the clove temperature, and to fully hydrate and activate nematodes that have been in anhydrobiosis on dried bulbs and cloves. Apparently, any benefits of the warming dip are achieved within 30 minutes' duration, and no further benefit is gained by extending the presoak time.

These studies were made on very heavily infected seed clove lots. The partial control achieved by hot-water dips without additives could be useful for lightly infected seed clove lots used in conjunction with efforts to produce nematode-free seed cloves, particularly where additives may not be available. These results confirm previous studies showing the lack of effective control by hot-water dips within the thermal tolerance range of garlic cloves.

Sodium hypochlorite is widely used for general surface disinfection and is toxic to nematodes by in vitro exposure to low concentrations (<1%) for short durations (≤ 7 minutes) (4). However, there has been little examination of NaOC1 as a dip additive for nematode disinfection of planting stock; a preliminary assessment on D. *dipsaci* disinfection in daffodil bulbs gave inconsistent results (12). Our studies have shown that aqueous solutions of sodium hypochlorite as the 20-minute hot-dip (49 C) treatment stage were consistently effective in controlling clove-borne *D. dipsaci* in four experiments. Concentrations of 1.052% to 2.63% gave effective control even when heavily infected cloves were treated and planted. Although treatments with NaOC1 were not quite as effective as hot water-formalin dip and not eradicative, they provide a level of control much higher than hot-water dip without additives.

Based on these results, sodium hypochlorite could be considered for commercial disinfection programs, especially when coupled with efforts to produce nematode-free planting stock. Solutions of 1.052%and 1.313% NaOC1 were as effective as those of higher concentration and were not phytotoxic to garlic. Higher concentrations (1.75-2.63% NaOC1) were phytotoxic to garlic weakened by heavy infection of *D. dipsaci,* but such concentrations are not necessary to achieve control. Our results could be used to support registration of sodium hypochlorite as a hotwater dip additive for nematode disinfection of garlic, and suggest investigation of nematode disinfection potential on planting stocks of other crops.

Effective control of *D. dipsaci* was achieved with abamectin at concentrations of 10-20 ppm used as a 20-minute cooldip stage following the standard warmingdip (30 minutes in water at 38 C) and hotdip (20 minutes in water at 49 C) stages. These treatments were not phytotoxic to garlic. Abamectin treatments were not quite as effective as hot-water formalin dip and did not eradicate *D. dipsaci* in experiments with heavily infected cloves, but they provided a much higher level of control than hot-water dips without additives. A shorter cool-dip duration of 10 minutes was less effective.

Abamectin as the hot-dip stage was less effective than when it was used as the cool dip. The nematode toxicity potential of abamectin may be adversely affected by heat, or the subsequent cool dip could act as a rinse and dilute any residual action of abamectin remaining on and in the garlic cloves. In support of the latter idea, although higher than expected numbers of active *D. dipsaci* were extracted from abamectin-treated cloves immediately after clove treatment and drying, the treatments proved highly efficacious when grown out in field plots. Thus, residual potency and delayed action of abamectin on nematode viability and infectivity could have been important factors contributing to overall treatment efficacy. Abamectin causes paralysis in nematodes by increasing membrane permeability to chloride ions in the neuromuscular system (11,15). Thus, abamectin toxicity action on *D. dipsaci* may progress beyond the actual time of exposure during dip treatment.

Our results constitute the first demonstration that an avermectin (abamectin) could have commercial application in nematode control for disinfection of planting stock. Previous studies of avermectin B1 as a nematicidal agent applied in irrigation water for nematode control showed variable efficacy (5,16,17). However, our findings could be used in support of registration of abamectin for garlic disinfection programs, and for justifying the study of abamectin nematode disinfection potential on planting stocks of other crops. Abamectin used in the final, cool-dip stage would have the advantage of reducing the volume of abamectin solution requiring disposal. Abamectin (avermectin B1) is used primarily in agriculture as a miticide on several tree and vegetable crops, cotton, and strawberries (11). Abamectin degrades rapidly by photooxidation, binds tightly to soil, and has a half-life in soil of 20-40 days, suggesting it would be unlikely to move to groundwater (11). A derivative of abamectin, ivermectin, is used as a systemic antiparasitic agent against endoparasites and ectoparasites of mammals and human filarial worm infections (11). These considerations suggest that abamectin may be compatible with and relatively safe for disinfection protocols of planting stocks.

In summary, these studies have further defined the limitations of hot-water dip treatments without additives that give only partial control of *D. dipsaci* at temperature and duration combinations that do not injure garlic seed cloves. More significantly, solutions of 1.052-1.313% sodium hypochlorite as a hot dip and 10-20 ppm abamectin in water as a cool dip following hot-water treatment are not phytotoxic to garlic and are effective in control of D. *dipsaci* clove-borne infections. Both are potential alternatives to replace the standard hot water-formalin dip disinfection protocol if used in programs aimed at producing nematode-free planting stock.

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