Efficacy of Chlorine for Decontaminating Water Infested with Resting Spores of *Plasmodiophora brassicae*

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ABSTRACT

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Irrigation water artificially infested with 1×10^6 resting spores of *Plasmodiophora brassicae* per milliliter was exposed to NaOCl concentrations of 0, 0.2, 2, 20, and 200 mg Cl/L. Resting spores also were exposed to the equivalent of 20 mg Cl/L for intervals of 0, 1, 5, 10, 30, 60, 180, 360, 720, and 1,440 min. Chlorine was effective in reducing the incidence of clubroot of cabbage at concentrations as low as 2 mg Cl/L when exposure time was at least 5 min. In field trials, clubroot incidence was reduced significantly by treatment of infested irrigation water with 200 mg Cl/L compared with the inoculated control; however, treatments with 200 mg Cl/L also significantly reduced plant height, fresh weight, and stand count. More information about chlorination and its influence on plant growth is required before chlorine can be used effectively to control *P. brassicae* in irrigation water.

Additional key words: chemical control, irrigation water treatment

Plasmodiophora brassicae Wor., the causal agent of clubroot of cabbage (*Brassica oleracea* var. *capitata* L.), was first described by Woronin in 1877 (22). Since then, clubroot has been regarded as one of the most important diseases of cruciferous crops in the world (9). There is no single consistent control measure of clubroot presently available (6,10).

Resting spores of *P. brassicae* present in irrigation water sediment may be resuspended in water by turbulence (5). If infested water is used for irrigation, the pathogen could contaminate noninfested seedbeds and fields.

Chlorine is a very good germicidal agent that has long been used to prevent the spread of waterborne human pathogens in public water supplies (13–15). Chlorine also has been used to control postharvest diseases and rots of vegetables and fruits (1,7,8,17,19). It has not been used extensively to treat irrigation water infested with plant pathogens (18). The purpose of this study was to determine if a practical chlorine treatment could be developed to disinfest water containing *P. brassicae* resting spores. A portion of this work has been reported (4).

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MATERIALS AND METHODS

Inoculum preparation, soil mixture, plant propagation, and clubroot rating index. Fresh, galled roots of *B. oleracea* var. *capitata* cv. Market Prize were field-collected and stored at −20 C. Resting spores were extracted, counted, and stored as described by Williams (21). Inoculum was prepared as described by Datnoff et al (5).

Steamed Groseclose silt loam soil and steamed Weblite (Weblite Corp., Blue Ridge, VA) were mixed together in equal volumes (v/v) with a motorized cement mixer. This soil mixture (87 cm^3) was amended with two soluble fertilizers: 29 g of slow-release Osmocote type 14 (Sierra Chemical Co., Milpitas, CA) and 29 g of immediately available Vance's 4-9-3 (Vance Co., Inc., Chilhowie, VA).

Market Prize cabbage plants were grown from seed (Joseph Harris Co. Inc., Rochester, NY) as described by Datnoff et al (5); however, the silt loam soil-Weblite (LSW) mixture was used in this study instead of the sediment-Weblite (SW) mixture (5).

Cabbage plants were rated for symptom intensity on a scale of 0–3, where 0 = no clubs, 1 = clubs on taproot, 2 = clubs on secondary root, and 3 = clubs on taproot and secondary roots (5). Disease incidence was calculated as a percentage of diseased plants in the population.

Preparation, amperometric titration, and pH of chlorine solutions. A commercially available sodium hypochlorite solution, 5.25% (w/v), contains 25 mg/ml of total chlorine (Cl) as determined with an amperometric titrator (model 17T1010, Fischer and Porter Co., Richmond, VA). Dilutions were made in sterile-distilled water to achieve concentrations of 0, 0.2, 2, 20, and 200 mg Cl/L. When chlorine exists as HOCl or OCl^- and in chemical combination with ammonia or organic nitrogen compounds, chlorine is defined as free and combined available chlorine, respectively. Total chlorine is defined as the summation of free and combined chlorine species (3,20).

Because pH is related to the species of free and combined chlorine present in a sample, the pH of each chlorinated sample was determined before and after incubation with resting spores with a Beckman Zeromatic SS-3 pH meter (Beckman Instruments, Inc., Fullerton, CA).

Effect of chlorination on resting spores in water. The effect of total chlorine on the ability of resting spores of *P. brassicae* to incite clubroot in cabbage seedlings was tested by treating suspensions of spores (1×10^6 spores per milliliter) in total chlorine concentrations of 0, 0.2, 2, 20, and 200 mg Cl/L at 25 C in darkness for 24 hr. Before and after incubation, spore concentration, pH, and chlorine concentrations (total, free, or combined) were determined. Twenty 28-day-old cabbage seedlings were suspended in various concentrations of chlorine-treated spore suspension in darkness for 36 hr at 25 C with a flotation device as described by Datnoff et al (5). After treatment, cabbage seedlings were removed from the flotation device, potted, and placed in the greenhouse under incandescent lights (8,250 lux at the soil surface) for 24 hr/day. Thirty days after planting, the cabbage plants were rated for root-clubbing.

Length of exposure time to chlorine and incidence of clubroot. To determine when chlorine is sporicidal, resting spores of *P. brassicae* were exposed in a solution containing 20 mg Cl/L for various lengths of time. Resting spores (10^6 spores per milliliter) in 250-ml flasks were exposed for 0, 1, 5, 10, 30, 60, 180, 360, 720, and 1,440 min at 25 C in the dark. After treatment, resting spores were washed twice onto a 1-μm filter (142 mm diameter, EAWP 142 00 filter, Millipore Corp., Bedford, MA) with 225 ml of distilled water by using a Manostat

Varistaltic Pump (Fisher Scientific Co., Richmond, VA). Impacted spores were washed from the filter and resuspended in 100 ml of distilled water. Fourteen-milliliter samples of the treated and washed spore suspensions were incubated with 21-day-old seedlings at 25 C in the dark for 36 hr. Seedlings were potted in LSW after incubation and placed in the greenhouse as described previously. Thirty days later, the cabbage roots were rated for clubbing.

Field trial. Microplots consisting of bottomless plastic pots 15 × 15 cm containing a Frederick silt loam soil (pH not determined) were seeded with about 30 Scarlet Knight radish (*Raphanus sativus* L.) seeds on 4 May 1982. Each row of five microplots was a plot. Microplots were buried 12.5 cm deep at 15-cm intervals; 40 cm of fallow soil separated each row. Five treatments were applied randomly within each of 10 replicate plots on 14 May 1982.

Suspensions were prepared to yield 10^6 resting spores per milliliter of distilled water in 500 ml, and appropriate amounts of chlorine were added to obtain 2, 20, and 200 mg Cl/L as described previously. Five minutes after treatment, 500 ml of the suspensions were dispensed into the microplots. Two controls consisting of untreated resting spores and distilled water alone were included. Treatments were evaluated for disease incidence, fresh weight, and stand count from all the plants in a microplot and for plant height from five randomly selected plants 35 days later.

Analysis of data. The pH level data were analyzed by Student's standard *t* test. Other data were analyzed by analysis of variance and Fisher's least significant difference procedures at $P = 0.05$. Each experiment was repeated at least once.

RESULTS

Laboratory. Estimated total chlorine levels were very close to the total chlorine levels determined by titration (Table 1). The proportions of free and combined chlorine increased as the concentration of total chlorine increased. The pH was not significantly different before or after incubation at each chlorine level, indicating chlorine concentrations were not altered during treatment of spores (Table 1).

Resting spore concentration, symptom intensity, and disease incidence all decreased as chlorine level increased (Table 2). The only disease occurred when chlorine concentration was less than 2 mg Cl/L after treatment for 24 hr. The resting spores exposed to 200, 20, and 2 mg Cl/L appeared devoid of cellular contents or coagulated even though the cell walls remained intact. Chlorine was phytotoxic and caused stunting and interveinal chlorosis to cabbage seedlings incubated 36 hr in treated, unwashed spore suspensions of 20 and 200 mg Cl/L. No treatment and a 1-min exposure of *P. brassicae* resting spores to 20 mg Cl/L caused a 73.3 and 66.6% incidence of clubroot, respectively. No cabbage plants developed clubroot symptoms after a minimum 5-min exposure.

Field. Only the 200-mg Cl/L treatment significantly reduced the incidence of clubroot compared with the inoculated control treatment (Table 3). The extent of disease was negligible for this treatment and was not significantly greater than the uninoculated control treatment. Chlorine treatment at concentrations at or above 20 mg Cl/L significantly decreased plant height, fresh weight, and the stand count compared with either the inoculated or uninoculated control treatments.

DISCUSSION

The results demonstrated that chlorine is probably sporicidal to *P. brassicae* resting spores, because resting spore concentrations were reduced and no

Table 1. Estimated and titrated chlorine levels and mean pH

Estimated chlorine levels (mg Cl/L)	Titrated chlorine levels[a] (mg Cl/L)			pH[b]	
	Free	Combined	Total	Before	After
200.0	140.00	59.00	199.00	10.5	10.8
20.0	10.59	4.43	15.02	9.1	8.9
2.0	0.15	2.27	2.42	6.9	7.1
0.2	0.02	0.27	0.29	6.5	6.8
0.0	0.00	0.00	0.00	6.4	6.4

[a] Chlorine levels measured with an amperometric titrator.
[b] Mean pH chlorine treatments before and after 24 hr of incubation with *Plasmodiophora brassicae* resting spores. Means of before and after measurements were not statistically significantly different for pairwise comparisons of chlorine level based on Student's standard *t* test at $P = 0.05$. Data are mean pH of three replicates.

Table 2. Resting spore concentration, symptom intensity rating, and disease incidence of clubroot in cabbage plants (*Brassica oleracea* var. *capitata* cv. Market Prize) exposed to *Plasmodiophora brassicae* resting spores treated with different levels of chlorine from NaOCl

Chlorine levels[v] (mg Cl/L)	Resting spore concentration[w] (spores × 10^5/ml)	Symptom intensity rating[x]	Disease incidence[y] (%)
200.0	3.9 a[z]	0.0 a	0.0 a
20.0	4.7 b	0.0 a	0.0 a
2.0	7.5 c	0.0 a	0.0 a
0.2	8.4 d	1.6 b	80.0 b
0.0	8.8 d	2.9 c	95.0 c

[v] Chlorine concentrations were prepared from a stock solution of 5.25% NaOCl (liquid bleach).
[w] Spores (1×10^6/ml) were treated at 25 C for 24 hr.
[x] Symptom intensity rating index: 0 = no clubs, 1 = clubs on taproot, 2 = clubs on secondary root, and 3 = clubs on the taproot and secondary roots.
[y] Disease incidence was calculated as a percentage of diseased plants in the population.
[z] Means followed by the same letter are not significantly different at $P = 0.05$ according to Fisher's least significant difference procedure. Data are means of four replicates.

Table 3. Incidence of clubroot, plant height, fresh weight, and stand count of radish seedlings (*Raphanus sativus*) grown in the field after treatment with chlorine (as NaOCl) and *Plasmodiophora brassicae* race 6

Treatment[w]	Disease incidence[x] (%)	Plant height (cm)	Fresh weight/plot (g)	Stand count[y] (no./plot)
Chlorine (2 mg Cl/L)	96 a[z]	5.9 bc	61.3 a	27 a
Chlorine (20 mg Cl/L)	93 a	5.7 c	46.3 a	24 a
Chlorine (200 mg Cl/L)	2 b	3.6 d	8.8 b	11 b
Inoculated control	90 a	7.4 a	74.9 a	26 a
Uninoculated control	4 b	7.1 ab	56.3 a	25 a

[w] Chlorine treatments plus 1×10^6 resting spores per milliliter in 500 ml, inoculated control = 1×10^6 resting spores per milliliter in 500 ml of distilled water, and uninoculated control = 500 ml of distilled water alone.
[x] Disease incidence was calculated as a percentage of diseased plants in the population.
[y] About 30 Scarlet Knight radish seeds were planted per plot.
[z] Means followed by the same letter are not significantly different at $P = 0.05$ according to Fisher's least significant difference procedure. Data are means of 10 replicates.

clubroot symptoms occurred when plants were exposed for 36 hr to spores treated with 2, 20, and 200 mg Cl/L. In addition, 20 mg Cl/L was sporicidal within a 5-min exposure in vitro. Phillips and Grendahl (12) and Segall (16) also demonstrated that chlorine is sporicidal to *Alternaria tenuis* and *Monilina fructicola*, respectively.

The results of the field study indicate that chlorine can be an effective treatment for reducing clubroot incidence that results from the use of *P. brassicae*-infested water. The chlorine treatments, however, were phytotoxic at 200 mg Cl/L, reducing plant height, fresh weight, and stand count. Chlorine damage also has been observed on other crops (2). This phytotoxic effect may be neutralized with such materials as Fe+, Mn+, NO_2, H_2S, or various organic compounds before treated irrigation water can be applied to transplants (3,20).

On an individual water source basis, chlorination efficiency would have to be determined before these results could be used effectively under field conditions. For example, many variable components of water exist, such as pH, inorganic and organic materials, and bacteria and other organisms that have an affinity for chlorine and reduce its effectiveness as a disinfectant (11,13,15). Inorganic and organic soil particles also could protect resting spores bound to them from contact with chlorine. Consequently, additional steps in water purification, such as filtration or sedimentation, might be required before use of chlorine is practical.

In conclusion, chlorine effectively reduced incidence of clubroot at relatively low chlorine concentrations under laboratory and field conditions. More information about chlorination is required, however, before chlorine treatment of irrigation water can be recommended for control of clubroot.

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