Evaluation of Disinfectant Seed Treatments to Reduce *Xanthomonas hortorum* pv. *carotae* in Carrot Seed Lots

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Introduction

Bacterial blight of carrot, caused by the plant pathogenic bacterium *Xanthomonas hortorum* pv. *carotae* (*Xhc*), is a common disease of carrot wherever the crop is grown. The disease can affect carrot foliage, stems, umbels, and roots and can be seed-borne. Symptoms of bacterial blight include small, irregular, chlorotic areas on leaves that can manifest into water-soaked, necrotic lesions. Lesions can also occur on stems and petioles. Floral infections can result in blighted umbels, reduced seed yield, and reduced germination rates of harvested seed. Once established, *Xhc* is difficult to control and disease prevention is challenging because *Xhc* is seedborne and seed treatments with hot water or disinfectants may not entirely eradicate the pathogen.

The seed-borne nature of *Xhc* makes it a major concern not only to the hybrid carrot seed industry in the Pacific Northwest but also to regions that import carrot seed for root production. Epiphytic populations can reach high levels on plants in the field, resulting in seed that is infected or infested by the pathogen. Seed lots that are highly infested with *Xhc* (>10^5 CFU/g seed) necessitate seed treatment to reduce the risk of bacterial blight occurring in commercial root crop production. Seed treatments are usually in the form of hot water treatment (52°C for 25 minutes) which can be effective but can reduce germination and/or shelf life of seed lots. Germination can be reduced further if seed lots need to be treated multiple times to reduce infestation levels below the 10^5 CFU/g threshold that was established for carrot seed planted in the Central Valley of California. Chemical seed treatments, which can remove bacterial pathogens that are borne on the seed surface, may provide alternative or additional methods for reducing *Xhc* in carrot seed lots. The objective of this project was to evaluate chemical disinfectants as seed treatments to reduce *Xhc* levels in carrot seed lots.

Materials and Methods

Teabags containing approximately 17 grams of commercially produced, naturally-infested seeds were subjected to one of the following 11 treatments: a non-treated control; hot water treatment (52° C for 25 min followed by a 60 s rinse under running tap water); SporeKill (ICA International Chemicals); PT81 (Ocion Water Sciences, Inc.); FT81 (Ocion Water Sciences, Inc.); KleenGrow (Pace 49 Inc.); Oxidate 2.0 (BioSafe Systems LLC); UpTake (Pace 49 Inc.); and bleach. Seeds were rinsed for 60 seconds under cool, running tap water immediately after treatments were applied with the exception of a non-rinsed, non-treated control and a non-rinsed SporeKill treatment. The non-rinsed SporeKill treatment was included to evaluate the effects of residual disinfectant on *Xhc* recovery and germination (*i.e.* phytotoxicity). Product rates, their active ingredient(s), and treatment times are listed in Table 1.

Treated seeds were air-dried on sterile paper towels in a laminar flow hood. Ten grams of the dried seed were put into 100 mL of phosphate buffer containing Tween and soaked for 2 hours at room temperature. Flasks were agitated on a horizontal shaker at 250 rpm for 5 minutes and the

seed wash was serially diluted to 10^{-5}. A total of 100 µl of each dilution was spread-plated onto two replicate plates containing semi-selective XCS medium. Plates were incubated in the dark at 28° C and the number of *Xhc* colonies was counted after 5 and 7 days. Colony counts were $\log_{10}$-transformed prior to analysis of variance (ANOVA) and pairwise comparisons were performed using Tukey's test. The experiment was arranged and analyzed as a randomized complete block design.

The effect of each treatment on seed germination was tested by plating 100 seeds from each treatment on sterile moistened filter papers placed in petri plates. The seeds were incubated for approximately 10 to 14 days at room temperature in the dark and the number of germinated seeds was counted. ANOVA and treatment comparisons were performed as described above.

Results and Discussion

Hot water treatment and SporeKill (rinsed and non-rinsed) significantly reduced the number of *Xhc* recovered compared to both non-treated controls (Table 1). Although the non-rinsed SporeKill treatment reduced *Xhc* levels to undetectable levels (limit of detection = 100 CFU/g seed), the treatment also reduced seed germination by 12 to 16% compared to the non-treated controls (Table 1). Rinsing the seed after treatment with SporeKill resulted in recovery of the pathogen and in increase in germination, suggesting that the product persisted on the seed after drying and influenced pathogen recovery and seed germination (Table 1). These results indicate that residual chemical disinfectants that remain on seed may reduce the recovery of *Xhc* and decrease seed germination. KleenGrow and UpTake, which contained the same active ingredient as SporeKill, did not significantly reduce seed germination but were also not as effective as hot water treatment or SporeKill at reducing *Xhc* in seed. In this study, hot water treatment provided the best control of *Xhc* in seed while still maintaining acceptable germination rates.

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Tables

Table 1. Effect of chemical disinfectant seed treatments on colony forming units (CFU) of *Xanthomonas hortorum* pv. *carotae* and germination (%) of carrot seed[1]

Treatment (rate)	Active ingredient(s)	Time	CFU/ gram seed	Germination
Non-treated, non-rinsed	**NA**	**NA**	2.96E+07 a	93% ab
Non-treated	**NA**	**25 min**	2.09E+07 a	97% a
Oxidate 2.0 (1%)	**hydrogen dioxide (27.1%); peroxyacetic acid (2.0%)**	**2 min**	1.66E+07 a	98% a
FT33 (1%)	**copper (4.16%); zinc (1.64%); sulfur (4.97%)**	**1 min**	4.99E+06 a	95% ab
PT81 (1%)	**copper sulfate pentahydrate (20.3%)**	**1 min**	4.95E+06 a	98% a
Bleach (1%)	**sodium hypochlorite (8.25%)**	**5 min**	2.11E+06 ab	96% a
UpTake (1%)	**didecyldimethyl ammonium chloride (7.5%); isopropanol (10.0%)**	**1 min**	8.14E+05 ab	92% ab
KleenGrow (1.16%)	**didecyldimethyl ammonium chloride (7.5%)**	**1 min**	1.15E+05 b	92% ab
SporeKill (1%)	**didecyldimethyl ammonium chloride (12%)**	**30 sec**	1.87E+02 c	88% ab
Hot water treatment	**NA**	**25 min**	1.75E+02 c	92% ab
SporeKill, non-rinsed (1%)	**didecyldimethyl ammonium chloride (12%)**	**30 sec**	0.00E+00 c	81% b
		***P*-value**	< 0.0001	0.0015

[1] All treatments were followed by a 60 s rinse under running tap water except where noted.