Evaluation of Copper and Quaternary Ammonium to Control Bacterial Blight in Steckling-Planted Carrot Seed Crops

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Abstract

Introduction

The Pacific Northwest, specifically central Oregon, central Washington, and Idaho's Treasure Valley, is a major global producer of hybrid carrot seed. Carrot seed crops are produced in direct-seeded (seed-to-seed) or steckling-planted (root-to-seed) fields. In the Pacific Northwest, stecklings used to produce root-to-seed crops are typically grown in California or Arizona and dug in January or February. Stecklings are stored at 1 to 2°C for 4 to 8 weeks to vernalize roots and condition them for bolting the following spring. Vernalization can be performed before or after the stecklings are shipped to seed producing regions.

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. Although severe symptoms of bacterial blight rarely occur in the semi-arid regions of the Pacific Northwest where carrot seed is produced, significant epiphytic populations can occur on asymptomatic plants in the field, resulting in seed that is infected or infested by 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.

In addition to infested seed, infected carrot stecklings may be an important source of inoculum for carrot seed producers in the Pacific Northwest. A previous study detected *Xhc* in 4 of 12 steckling crops that were sampled directly from shipping crates. Although the disease is often less prevalent in root-to-seed fields, outbreaks do occur. From 1931 to 1933, Kendrick reported bacterial blight outbreaks associated with infected stecklings in the Sacramento Valley of California. Similarly, during the 2014 season disease symptoms were observed in three steckling-planted fields which were well-isolated from other root-to-seed and seed-to-seed carrot seed crops (Jeremiah Dung, *personal observation*).

The use of disease-free seed and stecklings is an important component of an integrated disease management program to reduce the impact of bacterial blight on harvested seed. When possible, stecklings should be produced in areas where bacterial blight is not endemic. In addition, potentially infected stecklings should be treated prior to transplanting. However, there is a lack of effective control options for infected stecklings. The objective of this research is to evaluate potential treatments for stecklings infected with *Xhc* to prevent bacterial blight in root-to-seed carrot seed crops.

Materials and Methods

The effect of selected treatments on epiphytic populations of *Xhc* and bacterial blight was evaluated in a greenhouse. Carrot stecklings were obtained from commercial steckling production fields and vernalized according to standard industry practices. Each experimental unit consisted of three stecklings (subsamples). Stecklings were treated with KleenGrow (7.5% didecyldimethylammonium chloride; PACE 49 Inc., Canada), OCION PT81 (20.3% copper sulfate pentahydrate; OCION Water Sciences Group, Canada), OCION FT33 (4.16% Cu, 1.64% Zn, and 4.97% S; OCION Water Sciences Group, Canada), and/or ManKocide (15% mancozeb, 46% copper hydroxide; Certis USA, Columbia, MD) (Table 1). A non-treated/non-inoculated control and a non-treated/inoculated control were also included.

Stecklings were planted in potting mix and *Xhc* inoculations were performed using a CO_2-pressurized backpack sprayer. Stecklings were inoculated with a mixture of three *Xhc* isolates that were previously shown to cause bacterial blight on carrots under greenhouse conditions. Each steckling was inoculated with a total of 10^6 CFU/steckling. The non-inoculated control was mock-inoculated with sterile phosphate buffer (0.0125 M). Plants were arranged under a greenhouse bench covered with Remay cloth for 5 days to promote environmental conditions that are conducive to *Xhc* growth and epiphytic colonization.

Bactericide treatments were applied 5 days after *Xhc* inoculations. Bactericide treatments consisting of two applications were applied at 5 and 12 days post-inoculation. Bactericide treatments were applied using a CO_2-pressurized backpack sprayer calibrated to apply the products in 50 or 100 gallons/acre at 20 psi. The experiment was designed as a randomized complete block design with four replications.

Severity of bacterial blight symptoms was evaluated weekly after inoculation using a scale of 0 to 5 where: 0 = no symptoms, 1 = a few small lesions on one leaf, 2 = 5-10 lesions on one or two leaves, 3 = at least two leaves with prevalent symptoms, 4 = three or more leaves with extensive lesions, and 5 = >50% of the leaves with symptoms. Individual plants (subsamples) were destructively sampled and assayed at approximately 3- , 6- and 11 weeks after the first bactericide treatment application. Plants were finely chopped and a subsample of tissue was placed in a sterilized flask containing 250 ml of sterilized phosphate buffer (0.0125 M). Flasks containing buffer and foliage were incubated for 2 h, shaken for 5 min at 250 rpm on a gyratory shaker, and the rinsate from each flask was diluted serially up to 10^{-5}. Two 100 µl aliquots of each dilution were spread onto semi-selective XCS agar medium. Plates were incubated at 28^{o}C for 5 to 7 d. The number of colony forming units (CFUs) of *Xhc* from each plate was used to calculate the mean number of CFUs/ml. The chopped and rinsed foliage of each subsample was dried at 60^{o}C for at least 4 d and weighed to calculate the mean number of CFUs/g dry foliage. Data was subjected to analyses of variance (ANOVA) and means comparisons using Fisher's protected LSD.

Results and Discussion

Although the non-treated and inoculated control treatment exhibited the highest disease rating at the conclusion of the trial, relatively low bacterial blight severity was observed among all treatments (Table 1). None of the treatments significantly reduced *Xhc* populations compared to the non-treated and inoculated control (Table 1). The largest *Xhc* populations were observed on plants treated with a single application of ManKocide (1.91×10^8). *Xhc* was not detected on plants treated with two applications of KleenGrow+Ocion PT81 or on the non-treated/non-inoculated control plants. Lower populations of *Xhc* were detected on plants treated with Ocion PT81 (6.70×10^2) and two applications of KleenGrow+Ocion FT33 (8.47×10^3), but these

treatments were not significantly different than the non-treated/inoculated control. Phytotoxicity was not observed in any treatments and treatments did not prevent or delay bolting.

Acknowledgements

The researchers would like to thank PACE 49 Inc., OCION Water Sciences Inc., and Seminis Vegetable Seeds for providing funding and in-kind support.

Table 1. Bacterial blight disease ratings and populations of *Xanthomonas hortorum* cv. *carotae* (*Xhc*) on carrot foliage following treatments for bacterial blight control in carrot stecklings

Treatment	Rate	Disease rating (0-5)	*Xhc* CFU/g[1]
Non-treated and non-inoculated (control)	**NA**	0.00	0.00E+00 a
Non-treated and inoculated (control)	**NA**	2.00	2.43E+06 ab
ManKocide	**2.5 lb/acre**	1.75	1.91E+08 c
Ocion PT81	**40 oz/acre**	0.50	6.70E+02 a
Ocion FT33	**40 oz/acre**	0.75	6.87E+05 bcd
KleenGrow	**25 oz/acre**	0.00	2.46E+05 ac
KleenGrow + Ocion PT81	**25 oz/acre**	0.00	3.30E+06 bce
KleenGrow + Ocion FT33	**25 oz/acre**	0.25	2.41E+05 ac
ManKocide (x2, 7 day interval)	**2.5 lb/acre**	0.00	5.51E+05 a
Ocion PT81 (x2, 7 day interval)	**40 oz/acre**	0.00	1.54E+04 ade
Ocion FT33 (x2, 7 day interval)	**40 oz/acre**	0.00	2.44E+06 ade
KleenGrow (x2, 7 day interval)	**25 oz/acre**	0.25	4.20E+06 ade
KleenGrow + Ocion PT81 (x2, 7 day interval)	**25 oz/acre**	1.00	0.00E+00 a
KleenGrow + Ocion FT33 (x2, 7 day interval)	**25 oz/acre**	0.00	8.47E+03 a
		***P* = 0.06**	***P* = 0.02**

[1] Data were combined from the three sampling periods and log-transformed for ANOVA.