Evaluation of ManKocide Alternatives for Bacterial Blight Control in Steckling-to-Seed Carrot Seed Crops

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Introduction

Management of bacterial blight in carrot seed crops can be difficult and begins with the planting of healthy or treated seed. However, planting healthy or treated seed may not prevent the disease in biennial seed production fields because new plantings are often located adjacent to or in close proximity to the previous years' plantings. The pathogen, *Xanthomonas hortorum* pv. *carotae* (*Xhc*), is readily disseminated by contaminated irrigation water, wind-blown rain, insects, soil or carrot refuse and newly emerged seedlings of the next biennial season can become infected from nearby fields of the previous biennial season that already harbor *Xhc*. The disease has even been observed in isolated plantings, suggesting long distance dissemination of the pathogen on aerosolized seed crop residues or introduction of the pathogen on seedborne inoculum.

In addition to infested seed, infected carrot stecklings may be a source of inoculum for carrot seed producers. A previous study detected *Xhc* in 4 of 12 steckling crops that were sampled directly from shipping crates (du Toit et al. 2005). The use of disease-free stecklings is an important component of an integrated disease management program to reduce the impact of bacterial blight on harvested seed. However, there is a lack of effective control options for infected stecklings.

Carrot seed producers would like to reduce *Xhc* populations on harvested seed in order to minimize the need for hot water treatment and lessen the impact of bacterial blight on subsequent root crops in California, Washington, and other carrot-producing states and countries. Copper-based bactericides such as ManKocide (mancozeb + copper hydroxide) are applied multiple times each season to manage bacterial blight and increase seed quality, and are currently a primary control measure for bacterial blight in carrot seed crops. However, copper-based bactericides are most effective when used as preventative treatments and have limited ability to reduce *Xhc* populations once the pathogen becomes established in a seed crop (du Toit and Derie 2008).

The objective of this research is to evaluate potential post-plant treatments for stecklings harboring *Xhc* and evaluate potential alternatives to ManKocide for in-season control of *Xhc* in the field.

Materials and Methods

Plot establishment. A field trial was established at the Central Oregon Agricultural Research Center consisting of treatment plots 25 ft in length with 30 inch row spacing and 5 ft buffers in between plots. Female carrot stecklings were obtained from commercial steckling production fields and vernalized according to standard industry practices. Subsamples of stecklings were assayed for *Xhc* prior to planting and the pathogen was not detected. Stecklings were hand-planted approximately 6 inches apart in each row on April 12. Stecklings were rolled and watered in using overhead irrigation and then drip-irrigated for the remainder of the season. Standard management practices for steckling-to-seed hybrid carrot seed crops were followed.

In order to promote uniform infection in the plots, stecklings were inoculated on April 28 with a mixture of three *Xhc* isolates that were previously shown to cause bacterial blight on carrots under greenhouse conditions. *Xhc* inoculations were performed in-furrow using a CO_2-pressurized backpack sprayer. Each steckling was inoculated with a total of 10^6 CFU/steckling. The non-inoculated control was mock-inoculated with sterile phosphate buffer.

Treatments. Treatments included labeled and half-rates of 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), Oxycom Calcium (20% K_2O, 14% Ca, 7% S, and 4% P_2O_5; Redox Chemicals, LLC) and tank-mixes of KleenGrow + OCION PT81, KleenGrow + OCION FT33, and Oxycom Calcium + ManKocide (15% mancozeb, 46% copper hydroxide; Certis USA, Columbia, MD) (Table 1). A non-treated/non-inoculated treatment, a non-treated/inoculated treatment, and a ManKocide treatment were included as controls. Bactericide treatments were applied in a 6-inch band after planting using a CO_2-pressurized backpack sprayer calibrated to apply the products in 50 to 100 gallons/acre (Table 1). Initial bactericide treatments were applied on May 3 and 4. Subsequent in-season applications were made approximately every 2 weeks until the month of July, when bees were present for pollination of seed crops.

Disease evaluations. Incidence and severity of bacterial blight was rated at the onset of symptoms (June 1) and every 3 to 4 weeks thereafter. The incidence of bacterial blight was determined by counting the number of plants exhibiting bacterial blight symptoms in each plot. Bacterial blight severity was assessed on 10 randomly selected plants/plot using a scale of 0 to 5 where: 0 = no symptoms, 1 = a few small lesions on one leaf, 2 = 5 to 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. A disease index value was calculated by multiplying incidence and severity values for each plot.

Samples of foliage (10 leaves taken from 10 different plants in the center of each plot) were randomly collected and assayed for *Xhc* on May 5, June 2, June 23, and August 1, 2016. Foliage was chopped finely and a subsample was placed in sterilized phosphate buffer. Flasks containing buffer and foliage were incubated for 2 h and the rinsate from each flask diluted serially up to 10^{-5} and plated onto semi-selective XCS medium. Plates were incubated at 28° C for 5 to 7 days and the number of colony forming units (CFUs) of *Xhc* was determined. The chopped and rinsed foliage of each sample was dried at 60° C for at least 4 days and weighed to calculate the mean number of CFUs/g dry foliage.

Data analyses. The experiment was arranged as a randomized complete block design. CFU data was log-transformed and repeated measures data (CFU/g dry foliage and disease index) were converted to area under progress curves. Data were subjected to analyses of variance and multiple comparisons of treatments were made using Tukey's test.

Results and Discussion

Significant differences in pathogen populations (AUCFU) and disease incidence and severity (AUDPC) were observed between the non-treated/non-inoculated control and the non-treated/inoculated control. The ManKocide, Ocion PT81 (40 oz/acre), and KleenGrow (25 oz/acre) + Ocion PT81 (20 oz/acre) treatments all resulted in significantly lower AUDPC values than the non-treated/inoculated control but AUCFU values were not significantly different. AUDPC values were positively correlated with AUCFU values (r = 0.68).

Although ManKocide and ManKocide tank-mixed with Oxycom Calcium provided the best overall control, pathogen populations still reached relatively high numbers (> 10^6 CFU/g dry leaf tissue) at the mid-season evaluation date (June 23). It should also be emphasized that the application schedule used in this study (a total of 4 sprays spaced 2 weeks apart) is not representative of what can typically be performed under commercial production settings due to economic, environmental, and/or practical constraints.

The pathogen was not detected at the first sampling date (May 5), but *Xhc* increased in most plots as the season progressed, averaging 4.9 x 10^4 CFU/g on June 2, 4.5 x 10^5 CFU/g on June 23, and 2.0 x 10^8 CFU/g on August 1 (Fig. 1). High levels were observed on the non-treated/inoculated control at all three sampling dates, indicating that the artificial inoculations were successful. In contrast, the pathogen was not detectable on the non-treated/non-inoculated control until the final sampling date (August 1, 2016). Regardless, all treatments harbored *Xhc* at levels greater than 1 x 10^7 CFU/g dry leaf tissue at the final sampling date suggesting that pathogen populations can increase to large numbers during the pollination period.

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References

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Tables

Table 1. Effect of in-season foliar applications of bactericides, disinfectants, and a nutrient product on bacterial blight area under colony forming unit (AUCFU) and area under disease progress curve (AUDPC) values[1]

Treatment (rate)	Volume	AUCFU[2]	AUDPC[3]
Non-treated/non-inoculated (NA)	**NA**	145.2 a	0.0 a
Oxycom Calcium (2 lb/acre) + ManKocide (2.5 lb/acre)	**20 gal/acre**	214.4 ab	145.8 bc
ManKocide (2.5 lb/acre)	**50 gal/acre**	251.5 ab	106.4 b
KleenGrow (12.5 oz/acre) + Ocion FT33 (10 oz/acre)	**100 gal/acre**	336.0 ab	234.0 bc
Ocion PT81 (40 oz/acre)	**100 gal/acre**	358.9 ab	102.4 b
Ocion FT33 (40 oz/acre)	**100 gal/acre**	384.0 ab	257.3 bc
Ocion PT81 (20 oz/acre)	**100 gal/acre**	400.2 ab	200.8 bc
KleenGrow (25 oz/acre) + Ocion PT81 (20 oz/acre)	**100 gal/acre**	408.3 ab	88.6 b
Ocion FT33 (20 oz/acre)	**100 gal/acre**	415.8 ab	146.4 bc
KleenGrow (12.5 oz/acre)	**100 gal/acre**	438.8 bc	215.6 bc
Oxycom Calcium (2 lb/acre)	**20 gal/acre**	454.9 bc	306.5 bc
Non-treated/inoculated (NA)	**NA**	459.3 bc	562.4 c
KleenGrow (25 oz/acre)	**100 gal/acre**	475.6 bc	223.0 bc
KleenGrow (12.5 oz/acre) + Ocion PT81 (10 oz/acre)	**100 gal/acre**	489.9 bc	363.6 bc
KleenGrow (25 oz/acre) + Ocion FT33 (20 oz/acre)	**100 gal/acre**	480.6 bc	390.5 bc
	***P*-value**	0.0008	0.0063

[1] Treatments were applied approximately every 2 weeks.
[2] Area under colony forming unit (AUCFU) values were calculated from samples taken May 5, June 2, June 23, and August 1, 2016.
[3] Area under disease progress curve (AUDPC) values were calculated from disease index values obtained on June 1, June 24, and July 26, 2016.

Figures

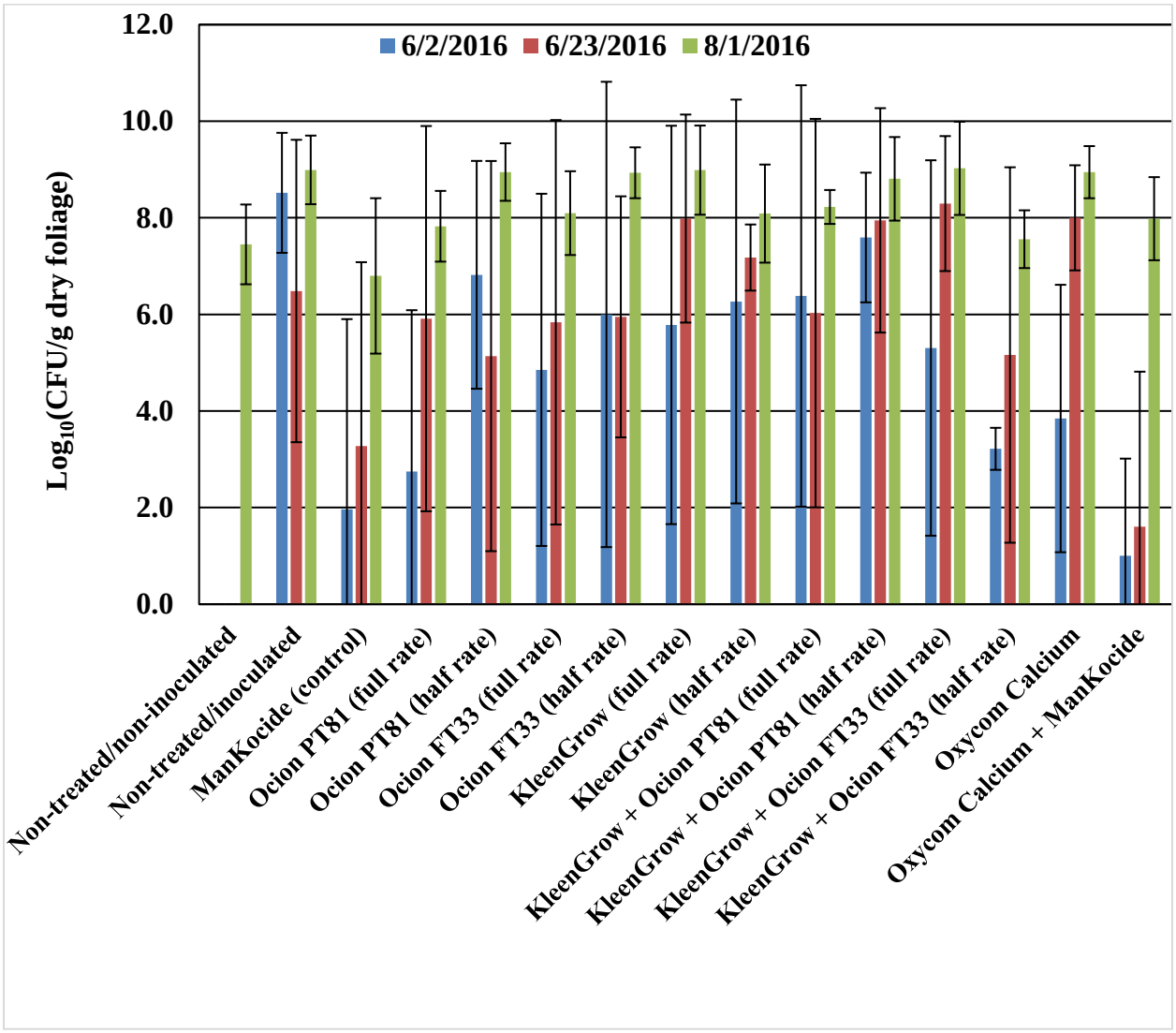

Fig. 1. Effect of bactericides, disinfectants, and a nutrient treatment on colony forming units (CFU) of *Xanthomonas hortorum* pv. *carotae* on carrot foliage at three different sampling dates. CFU values were $\log_{10}$-transformed. Error bars represent standard deviations.