CALIFORNIA FRESH CARROT ADVISORY BOARD RESEARCH
REPORT
March 1, 2018 – February 28, 2019

Project Title: Evaluation of Quaternary Ammonium Chloride Products for Bacterial Blight Control

Abstract:

Bacterial blight is an important disease of carrots in California production systems. The use of copper-based bactericides are a primary means for controlling bacterial blight in carrots and can be applied several times during the production season. Quaternary ammonium compounds (QACs) exhibit broad-spectrum antimicrobial activity and may provide new bactericide options for bacterial blight control. In addition, synergistic interactions between QACs and copper have been observed, potentially allowing for greater antimicrobial activity and improved control. The objective of this project was to evaluate quaternary ammonium chloride products, alone and in combination with ManKocide®, for control of bacterial blight in carrots. ManKocide® and KleenGrow®+ManKocide® treatments significantly reduced epiphytic *Xanthomonas* populations from the non-treated control at one of two treatment times. Phytotoxic effects were not observed, and no impacts on bolting frequency or timing were evident in treated plots.

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Statement of the Problem with Particular Reference to California Carrots:

Bacterial blight, caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*), is a common disease of carrot and is considered a major disease of carrots in California production (4). Copper products are the only available option for chemical management of bacterial blight and provide fair to good control, but have limited efficacy once *Xhc* populations become established on foliage. More research is needed to evaluate products and techniques for the control of bacterial blight in carrots (4).

Literature Review:

Bacterial blight, caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*), is an important seedborne disease of carrot. The bacterium can survive and reproduce epiphytically, causing disease symptoms when large populations are attained on foliage (> 10^6 CFU/g leaf tissue). Copper-based bactericides such as ManKocide® (mancozeb + copper hydroxide) are a primary control measure for bacterial blight in carrot seed crops and are often applied several times during carrot seed production to manage

bacterial blight. 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 (1).

When used alone, quaternary ammonium compounds (QACs), can exhibit broad-spectrum antimicrobial activity (3). QACs are thought to act on the phospholipid components of bacterial cell membranes, causing membrane deformation, leakage of low-molecular weight cytoplasmic material, and disruption of proton pumps. Research on *Pseudomonas aeruginosa*, a biofilm-producing opportunistic pathogen that is particularly resilient to chemical control, suggests that combinations of QACs and copper can act synergistically to provide broad-spectrum antimicrobial activity (2, 5). Ammoniacal copper quaternary (ACQ), a combination of the QAC didecyldimethyl ammonium chloride and copper oxide, has been used as a fungicidal and insecticidal wood preservative in the forest industry for over 20 years and is considered to be environmentally-friendly (2).

Preliminary research demonstrated that treatment with the QAC didecyldimethyl ammonium chloride alone was effective at reducing *Xhc* on carrot foliage, but more data is needed in order to pursue potential product registration for carrot and/or carrot seed crops. Research is also needed to evaluate combinations of QACs and copper for *Xhc* control in carrots. QACs, alone or with copper, may also provide control of important fungal diseases of carrot.

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3. McDonnell, G., and Russell, A. D. 1999. Antiseptics and disinfectants: activity, action, and resistance. Clinical Microbiology Reviews 12(1):147-179.
4. Participants, Workgroup. 2005. A Pest Management Strategic Plan for Fresh Carrot Production in California. June 2005.
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Research Objectives:

The objective of this project was to evaluate quaternary ammonium chloride products, alone and in combination with ManKocide®, for control of bacterial blight in carrots.

Materials and Methods:

Plots of a proprietary female hybrid carrot seed line were established at the Central Oregon Agricultural Research and Extension Center in Madras, OR on August 17, 2017. Treatment plots were 10 ft. in length with 5 ft. buffers in between plots and consisted of four rows of carrots spaced 30 in. apart. Plots were drip-irrigated and standard management practices for seed-to-seed hybrid carrot seed crops were followed.

Levels of *Xhc* were quantified from each plot on May 9 and June 7, 2018 prior to treatment applications using a serial dilution plating assay. Samples of foliage were randomly collected (one leaf was taken from each of 20 different plants in the center two rows of each plot) and chopped into pieces ≤ 0.5 in. in size. A subsample of chopped foliage was placed in 100 ml of sterilized 12.5 mM

phosphate buffer with one drop of Tween20, incubated for 2 h and then agitated for 5 min on a shaker. The rinsate from each flask was diluted serially up to 10^{-5} and plated onto semi-selective XCS medium. Plates were incubated at 82° F for 7 to 10 days and the number of colony forming units (CFUs) of *Xhc* was determined. The chopped and rinsed foliage of each sample was air-dried at 95°F and weighed to calculate the mean number of CFUs/g dry foliage.

Treatments were applied on May 10 and June 8, 2018 using a CO_2 backpack spray boom. The boom was outfitted with three TP8002VS flat fan nozzles spaced 18-in apart and pressurized to 28 psi. A total of five bactericide treatments were tested: SporeKill® (120 g/L didecyldimethyl ammonium chloride) at a rate of 2.5 oz./A; KleenGrow® (7.5% didecyldimethyl ammonium chloride, 10% isopropanol, and 2% ethanol) at a rate of 7.6 oz./A; ManKocide® (15% Mancozeb and 46.1% copper hydroxide) at a rate of 2.5 lbs./A; a tank-mix of SporeKill® and ManKocide®; and a tank-mix of KleenGrow® and ManKocide®. A non-treated control was also included. The experiment was arranged as a randomized complete block design with four replications per treatment.

Post-treatment foliage samples were taken on May 14 and June 11. Samples from each plot were subjected to the leaf wash assay described above to determine foliar *Xhc* populations after bactericide treatment. CFU data were treated as count data and analyzed at each sampling time using negative binomial regression.

Incidence and severity of bacterial blight was rated at the onset of symptoms and monthly thereafter. The incidence of bacterial blight was determined by counting the number of plants exhibiting bacterial blight symptoms in each plot. Bacterial blight severity will be 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. Phytotoxicity was evaluated using a 0-5 scale (0 = no phytotoxicity, 5 = dead plant). The impact of each treatment on bolting frequency and timing was also evaluated.

Results and Discussion:

The distribution of the pathogen among the naturally infested plots was variable on the first sampling date, ranging from 0 to 10^7 CFU/g leaf tissue (Table 1). *Xanthomonas* populations increased from 0 CFU to 10^9 CFU/g leaf tissue by the final sampling date in June. Despite the high epiphytic populations of *Xhc* in plots, bacterial blight symptoms were not observed.

Significant differences in epiphytic *Xhc* populations were not observed after the first treatment application in May, with pathogen populations ranging from 10^4 to 10^7 CFU/g leaf tissue among the plots. By the second treatment application, *Xhc* populations reached high levels (10^7 to 10^9 CFU/g leaf tissue) and all treatments showed reduced pathogen populations compared to the non-treated control; however, only ManKocide® and KleenGrow®+ManKocide® treatments were significantly different from the non-treated control (Table 1). It is notable that the second ManKocide® treatment reduced *Xhc* populations from 10^8 to 10^3 CFU/g leaf tissue, a 100,000-fold decrease. Phytotoxic effects were not observed, and no impacts on bolting frequency or timing were evident in treated plots.

Tables

Table 1. Effect of copper, quaternary ammonia, and tank-mix treatments on log-transformed colony forming units (CFU) of *Xanthomonas hortorum* pv. *carotae* on carrot foliage[z]

	CFU/g leaf tissue			
	Pre-treatment	**Post-treatment**	**Pre-treatment**	**Post-treatment**
Treatment	**5/9/2018**	**5/14/2018**	**6/7/2018**	**6/11/2018**
Non-treated control	0.00	4.44	9.08	9.02
ManKocide®	6.47	4.57	8.61	3.02*
KleenGrow®	7.81	7.64	7.89	7.67
KleenGrow® + ManKocide®	0.00	3.83	7.38	6.13*
SporeKill®	7.20	6.50	7.31	8.03
SporeKill® + ManKocide®	6.99	3.75	8.17	7.89
	***P* = 0.82**	***P* = 0.76**	***P* = 0.45**	***P* = 0.02**

[z] Treatments followed by an asterisk are significantly (*P* = 0.05) different than the non-treated control using negative binomial regression.