

Introduction

Bacterial leaf blight, caused by the bacteria *Xanthomonas hortorum* pv. *carotae* (*Xhc*), can be a significant problem in some carrot production areas in California. Symptoms appear as irregular brown spots on leaf margins and may appear water-soaked with an irregular yellow halo. With time, the yellow halo disappears, and the spots may coalesce and develop into dark brown streaks on petioles (Davis and Nunez, 2007). Bacterial oozing, a clear bacterial exudate, which is a diagnostic feature of this disease, can usually be seen with lesions on leaves and petioles. Bacterial blight is often noticed as brown areas within the field about 3-4 feet in diameter.

An effective management strategy for bacterial blight is to prevent the introduction of this disease into a field by planting disease-free or indexed seed (Gilbertson, 2002). However, once the pathogen has been introduced to a field, subsequent plantings may become infected as the bacteria can survive on infected plant debris from season to season. The pathogen can then be spread by splashing rain, irrigation, and insects to reinfest subsequent plantings of carrot seedlings. Thus management recommendations include practicing 2- to 3-year crop rotations, incorporating infected crop debris immediately after harvest (to hasten decomposition), avoiding sprinkler irrigation if feasible, and applications of copper-based bactericides (Gilbertson, 2002). However, copper-based bactericides are most effective when used as preventative treatments and have limited ability to manage the pathogen once the disease is established in the field (duToit and Derie, 2008). Also, sometimes it is not practical for growers to apply copper products every 2 weeks or on a frequent basis. Therefore, there is an opportunity to find alternative products with low cost, ease of application, and prolonged residual effects. The objective of this research was to evaluate various post-plant foliar treatments for in-season control of bacterial blight in carrot root crops.

Materials and Methods

A field trial was conducted at the Kern County Extension Research Station in Shafter, CA to evaluate the efficacy of various bactericide products. The trial was conducted as a randomized block design with four replications and ten treatments including a non-treated control. Each plot was comprised of two 30" wide beds that were 15 ft long with a 5 ft buffer between plots along the bed. Carrot seed of a fresh market variety naturally infested with *Xhc* (1.85×10^8 CFU/g seed) was seeded (three lines per bed) using the Jang JP1 seeder. Plots were sprinkler irrigated and the trial was managed following grower standard agronomic practices. Treatments were applied using a battery-operated operated backpack sprayer calibrated to deliver 40 gpa at 25 psi using a flat fan JSF11002 nozzle. Treatments were applied during the growing season on February 24, March 9, March 25, and April 13, 2022. Treatments and rates for each treatment are listed in Table 1.

Samples of foliage (approximately 100 leaves taken from 100 different plants in the center of each plot) were randomly collected and assayed for *Xhc* on March 8, March 14, March 30, and April 18) to assess the reduction in epiphytic *Xhc* populations due to each treatment.

Sample processing

Leaf samples were cut into 1 to 3 cm² pieces and subjected to a phosphate buffer wash assay (Scott and Dung, 2020). One ml aliquots were sampled from each leaf wash assay and treated with a DNA binding dye propidium monoazide (PMAxx™, Biotium, Fremont, CA, USA) (Temple et al, 2013). Following the PMAxx™ treatment, the samples were centrifuged for 3 min at 12,500 rcf after which the supernatant was discarded. Preparation of genomic DNA was obtained from this pelleted material through an InstaGene™ Matrix extraction (150 µl) according to the manufacturer's instructions and 1.5 µl of this DNA preparation was used as the template in the *Xhc*-specific qPCR assay described above. The leaf tissue was retained from the leaf wash assay, dried at 35°C for 5-7 days, and subsequently weighed. The level of *Xhc* in the field was calculated as *Xhc* genomes per gram leaf tissue dry weight based on the leaves that were sampled at each sampling date and the Area under the colony progress curve (AUCPC) was also calculated. qPCR data were log₁₀-transformed and subjected to analysis of variance (ANOVA) for each of the sampling dates, and multiple comparisons of the treatments were conducted using Dunnett's test.

Table 1: Treatments, rates, and active ingredients of various bactericide treatments applied in-season for *Xhc* control

	Treatment	Rate per acre	Active ingredient	Manufacturer
1	none			
2	Oxidate 5.0	0.5gal/50gal of water/A	Hydrogen peroxide 27% and Peroxyacetic acid 5%	BioSafe systems
3	Kocide 3000 O	1.5 lbs/A	Copper hydroxide 46.1%	Certis USA LLC
4	LifeGard	1.75 oz/A	Bacillus mycooides isolate 40%	Certis USA LLC
5	Actigard 50WG	0.75oz/A	Acibenzolar-S-methyl 50%	Syngenta
6	ChampION	1.5lbs/A	Copper hydroxide 46.1%	Nufarm Americas Inc.
7	Cueva	6.36 gal/A/ application	Copper octanoate 10%	Certis USA LLC
8	ManKocide	2.25lbs/A	15% mancozeb, 46% copper hydroxide	Certis USA LLC
9	Mastercop	1.5 pints/A	21.46% copper sulfate pentahydrate	Adama
10	Nordox	2.5 lbs/A	Cuprous oxide 56.4%	Nordox Industrier AS

Table 2. Log₁₀-transformed genomes of *Xanthomonas hortorum* pv. *carotae* on carrot foliage at four different sampling dates after applications of various bactericide treatments

Treatment	Foliage				AUCPU
	Sampling#1 (March 8)	Sampling#2 (March 14)	Sampling#3 (March 30)	Sampling#4 (April 18)	
Control	6.45	8.17	7.51	7.17	9.37
Oxidate	5.99	7.72	7.02	6.07	8.89
Kocide 3000 O	4.17*	7.44	5.58	4.39**	8.49*
LifeGard	5.23	7.19	6.46	5.86	8.35*
Actigard 50WG	5.87	7.48	5.84	6.22	8.56
ChampION	5.67	8.00	6.35*	4.29	9.06
Cueva	6.05	6.87	5.97	2.17**	8.00*
ManKocide	5.54	7.72	7.12	4.39	8.91
Mastercop	5.84	8.15	6.09	5.88	9.20
Nordox	5.70	6.84	3.59*	3.98*	7.89**
P value	0.197	0.2197	0.0762	0.005	0.0322

Treatments followed by the * are significantly different than the control at $\alpha=0.05$ using Dunnett's test
Treatments followed by the ** are significantly different than the control at $\alpha=0.10$ using Dunnett's test

Results

Compared to the non-treated control, *Xhc* populations were slightly lower on carrot foliage in all other treatments at all sampling dates (Table 2). Although some treatments such as Kocide and Nordox performed slightly better than others, the *Xhc* populations increased across all treatments ($>10^5$ CFU/g dry leaf tissue) in the second sampling. The pathogen levels decreased relatively in the third and final sampling. In the final sampling, treatment Cueva had the lowest pathogen population (1.47×10^2) followed by Nordox (9.52×10^3), and Kocide (2.46×10^4). These levels are lower than the levels of colonization ($>10^6$ CFU/g of leaf tissue) necessary for the development of symptoms in the field (Davis and Nunez, 2007).

Significant differences in the 'Area under colonization progress curve' (AUCPC) were observed with treatments Kocide, LifeGard, Cueva, and Nordox resulting in lower AUCPC values than the non-treated control. The treatment application schedule applying treatments every 14 to 20 days in the trial is not an ideal representation of the management practices in commercial carrot production due to cost-effectiveness and practicality of application.

References

- Davis, R.M., & Nunez, J. 2007. Integrated Approaches for Carrot Pests and Diseases Management. In: Ciancio, A., Mukerji, K.G. (eds) General Concepts in Integrated Pest and Disease Management. Integrated Management of Plants Pests and Diseases, vol 1. Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6061-8_6
- du Toit, L.J. & M.L. Derie. 2008. Effect of inoculum concentration and timing of ManKocide application on bacterial blight of carrot, 2007. Plant Disease Management Report 2:V008
- Gilbertson, R. L. 2002. Bacterial leaf blight of carrot. Pages 11-12 in: Compendium of Umbelliferous Crop Diseases. American Phytopathological Society, St. Paul, MN.
- Scott, J. C., & Dung, J. K. 2020. Distribution of *Xanthomonas hortorum* pv. *carotae* populations in naturally-infested carrot seed lots. Plant Disease. <https://doi.org/10.1094/PDIS-12-19-2674-RE>.
- Temple, T. N., du Toit, L. J., Derie, M. L., & Johnson, K. B. 2013. Quantitative molecular detection of *Xanthomonas hortorum* pv. *carotae* in carrot seed before and after hot-water treatment. Plant Disease, 97(12), 1585–1592.
- Umesh, K. C., Davis, R. M., & Gilbertson, R. L. 1998. Seed contamination thresholds for development of carrot bacterial blight caused by *Xanthomonas campestris* pv. *carotae*. Plant Disease. 82 (11):1271-1275.

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