Timing Effect of ManKocide® Applications on Bacterial Blight in Carrot Seed Crops in Central Oregon, 2008-2009

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Abstract

Two separate field trials, one with a seed-to-seed carrot seed crop and the other with a steckling-to-seed carrot seed crop, were set up to evaluate applications of ManKocide applied pre- and post-inoculation of carrot foliage with *Xanthomonas hortorum* pv *carotae* (*Xhc*) for suppression of bacterial blight in carrot seed crops. Inoculation of the plants with *Xhc* was done in fall 2008 and spring 2009 in the seed-to-seed trial and only in spring 2009 in the steckling-to-seed trial. The tissues of carrot plants (including leaves, stems, and petioles, as well as umbels and flowers after the latter had developed) were collected monthly and assayed for the population levels of *Xhc* during the cropping season, except in the winter (December 2008 to April 2009). The population levels of *Xhc* on the seeds harvested from each plot were also assayed. The results showed that applications of ManKocide did not significantly reduce the bacterial population on plants or on harvested seeds in the seed-to-seed trial. In the steckling-to-seed trial, applications of ManKocide did significantly reduce the bacterial population on the foliage, but the treatments did not significantly suppress the population of *Xhc* detected on the harvested seeds. Harvested seed from plots inoculated with *Xhc* were above the current threshold for commercial markets (10^4 to 10^5 colony-forming units (CFU)/g seeds) and would require hot-water treatment despite three to six field applications of ManKocide.

Methods and Materials

Field Experiment Design

Two separate trials were conducted at the Oregon State University Central Oregon Agricultural Research Center to evaluate the effect of the timing of applications of ManKocide on suppression of *Xhc* in seed-to-seed and steckling-to-seed carrot seed crops in central Oregon. A female carrot inbred line was used in both trials to avoid cross-pollinating other carrot seed crops in the region. Seed-to-seed trial plots were planted August 13, 2008. Carrots were planted at a 30-inch wide row spacing in long strips through the field. Strips were separated by a 20-ft wide alley not planted with carrots. Prior to applying the bactericide treatments, carrot plants were removed from 30-ft wide cross-alleys between plots within each strip. Thus, plots were 20-ft wide (8 rows) by 20-ft long, separated by 20-ft alleys between adjacent strips and 30-ft alleys between adjacent plots within each strip. The following nine inoculation- ManKocide treatment combinations were included in the trial with seed-to-seed carrots, with the treatments arranged according to a randomized block design with seven replicate blocks.

1. No *Xhc* inoculation, no ManKocide application
2. Fall *Xhc* inoculation, no ManKocide application
3. Fall *Xhc* inoculation, 3 ManKocide applications: 1 pre-inoculation + 2 post-inoculation
4. Fall *Xhc* inoculation, 2 ManKocide applications: both post-inoculation

5. Spring *Xhc* inoculation, no ManKocide application
6. Spring *Xhc* inoculation, 3 ManKocide applications: 1 pre-inoculation + 2 post-inoculation
7. Spring *Xhc* inoculation, 2 ManKocide applications: both post-inoculation
8. No *Xhc* inoculation, 6 ManKocide applications: 3 fall + 3 spring treatments
9. Fall and spring *Xhc* inoculations, 6 ManKocide applications: 3 fall + 3 spring treatments

The steckling-to-seed carrot plants were transplanted on April 8, 2009. The following five treatments were included in that trial:

1. No *Xhc* inoculation, no ManKocide application
2. Spring *Xhc* inoculation, no ManKocide application
3. Spring *Xhc* inoculation, 3 ManKocide applications: 1 pre-inoculation + 2 post-inoculation
4. Spring *Xhc* inoculation, 2 ManKocide applications: 2 post-inoculation
5. Spring *Xhc* inoculation, 1 ManKocide application: pre-inoculation

The five treatments were arranged according to a randomized complete block design with seven replicate blocks. Plot size and alleys between plots were as for the seed-to-seed trial. All plots were irrigated using furrow irrigation, and fertilized according to commercial standard practices. Honey bees were not used for pollination, but bees from a nearby hive were observed in the plots. Temperature and relative humidity were recorded using three HOBO data loggers in the top third of the canopy.

Inoculation of *Xhc* and Application of ManKocide

Fall inoculation was done on September 23, 2008 and spring inoculation on April 17, 2009. An isolate of *Xhc* that originated from carrots was cultured in liquid medium at room temperature for 24-48 hours and used to prepare a suspension of 10^2 *Xhc* CFU/ml in 0.0125 M phosphate buffer. Inoculation was conducted by applying the suspension of *Xhc* uniformly through the plots in 20 gal/acre using a standard CO_2-pressurized backpack sprayer. The 10-ft spray boom had 5 nozzles (8002 tips) that allowed coverage of 4 rows of carrot plants at a time. Two passes were made to cover all 8 rows in each plot at each application.

Pre-inoculation application of ManKocide was targeted for 4 days prior to inoculation of *Xhc*. Two post-inoculation applications of ManKocide were 3 and 10 days after inoculation of *Xhc*. Specifically, the pre- and two post-inoculation applications of ManKocide® for fall inoculations ocurred on September 19 and 26, and October 2, 2008, respectively. The three ManKocide applications for spring inoculation were applied on April 13, 20, and 27, 2009. At each application, ManKocide was applied at 2.5 lb product/acre using a commercially designed tractor-mounted spray tank and boom.

Sampling Methods and Assay for *Xhc*

For the seed-to-seed trial, samples were collected on October 7 and November 3, 2008, and again on May 11, June 15, July 15 and August 2, 2009. For the steckling-to-seed trial, sampling dates were June 1, July 21, August 10, and September 2, 2009. At each sampling date, carrot tissues (leaves, stems, petioles, and, when present, umbels and flowers) were collected from 30 plants/plot, packed in a clean plastic bag, and stored in a refrigerator until the samples were

assayed within 24 hours of collection. In the fall and early spring, when plants were small, all the foliage from each composited set of carrot plants was sampled/plot. In the spring and summer, as plants became larger and bolted, a representative amount of foliage, petioles, stems, and umbels was collected from each of the 30 plants/plot. From September 9 to 23, umbels were hand clipped from plots as they matured. A sample of 100 umbels representing a typical harvest maturity (mostly primary and secondary umbel orders) was collected from each plot and packed in a clean paper bag for further air drying in the laboratory. Hands and tools were disinfested between plots during sample collection. After 3-4 weeks of air drying, seeds were hand-rubbed from the umbels collected in each plot. Seeds were deburred and then passed through screens by hand, using standard research equipment mimicking the commercial combine-deburring and seed-cleaning processes. All tools, equipment, and hands were disinfested between samples at each step of the processes.

The carrot tissue samples collected were chopped into 1 to 4 mm pieces, soaked in a sterile flask with sterile 0.0125 M phosphate buffer, and shaken at 200 rpm on a gyratory shaker for 1 hour. The rinsate from each flask was diluted serially (10^{-1} to 10^{-8}). Under aseptic conditions, 100 µl aliquots of each dilution were spread onto XCS agar and incubated at 27.7777778^{o}C for 1 week. CFUs typical of *Xhc* were counted on each plate. The rinsed plant tissues were immediately dried in an oven at 55^{o}C, and the dry weight determined for each sample to calculate CFU/g dry tissue.

Each seed sample was soaked in 100 ml saline (0.85 percent NaCl) overnight at 4^{o}C. Two drops of Tween® 20 were then added to each flask and mixed by shaking on a rotary shaker for 5 min. A dilution series of the rinsate was spread onto XCS agar medium and CFUs counted after a week of incubation at 82^{o}F as described for carrot tissue samples

For each sample, total CFUs was divided by dry weight and then log-transformed to log CFU/g dry tissue or seeds. Analysis of variance (ANOVA) was then performed on log CFU/g dry weight using the General Linear Models procedure of SAS. Individual contrasts were calculated between non-inoculated plots and plots inoculated with *Xhc* in fall 2008 that did not receive ManKocide applications, between non-inoculated plots and plots inoculated in spring 2009 that did not receive ManKocide applications, between plots inoculated in fall with and without ManKocide applications, and between plots inoculated in spring with and without ManKocide applications.

Results and Discussion

Seed-to-seed Trial

Air temperature in the canopy ranged from 40 to 110°F and relative humidity ranged from 25 to 90 percent, with the highest average humidity recorded in July. Inoculation of the carrot foliage with *Xhc* in the fall was successful as the population levels of *Xhc* were higher in plots inoculated and not treated with ManKocide than in plots not inoculated and not treated with ManKocide, from October 7, 2008. Differences in *Xhc* counts were also significant from May 11, 2009 to the last sampling date before harvest, but not significant on the harvested seeds (Fig. 1, Tables 1 and 3). Foliar symptoms were not apparent until almost 3 months after the first

inoculation. Symptom severity/plot was not measured due to size of the plots and removal of plants during sampling. The populations of *Xhc* declined from October 7 to November 3 to very low levels for all plots regardless of treatment. Inoculation was also successful in the spring, when population levels of *Xhc* in plots without ManKocide applications were significantly higher in plots inoculated with *Xhc* than non-inoculated plots, from May 11 to the last sampling date. However, this difference in population was not significant in the harvested seeds (Fig. 1, Tables 1 and 3). Low levels of bacterial populations were detected in non-inoculated plots as early as October 7, 2008, suggesting natural inoculum was present in the area during the fall (Fig. 1 and Table 1).

Among those plots inoculated with *Xhc* in the fall, the ManKocide treatments had no significant effect on population levels of *Xhc* detected on October 7 or at any other sample date (Fig. 1, Tables 1 & 3). The ManKocide applications also did not significantly reduce the bacterial population in plots inoculated in spring either (Fig. 1, Tables 1 and 3). For plots not inoculated with *Xhc*, six applications of ManKocide (three in the fall and three in the spring) also did not significantly reduce the pathogen populations on carrot plants or seeds.

Non-inoculated plots showed an average population of 4.6 Log CFU/g seed, while the levels of bacterial populations in plots inoculated with *Xhc* were all above 6.0 Log CFU/g seed, except plots that received six applications of ManKocide, which was 4.1 Log CFU/g seed. All seed lots harvested from inoculated plots in this trial would have had to be hot-water treated to be commercially viable for sale to California (Table 1).

Steckling-to-seed Trial

Results of initial testing of 20 vernalized roots (stecklings) prior to transplanting showed that 0 out of 20 stecklings carried viable *Xhc*. Air temperature ranged from 40 to 100°F and relative humidity ranged from 25 to 80 percent in the canopy, with the highest average humidity recorded in July. It was interesting that in the non-inoculated plots that were not treated with ManKocide, populations of *Xhc* were lower in the steckling trial than in the corresponding plots in the direct-seeded trial (Fig. 1, Tables 1 and 2). This suggests that natural inoculum may have survived on the overwintered carrot plants and may be important in development of *Xhc* populations on carrot plants and developing umbels/seeds. In the steckling-to-seed trial, among plots not treated with ManKocide, the population of *Xhc* in inoculated plots was significantly higher than in non-inoculated plots for all sampling dates as well as on the harvested seeds, suggesting that inoculation was very successful (Fig. 1, Tables 2 and 3). In inoculated plots, applications of ManKocide significantly kept the populations of *Xhc* lower than those detected in plots not treated with ManKocide, for all the sampling dates, but the difference became smaller as the crop matured (Fig. 1 and Tables 2 and 3).

The ManKocide treatments showed insignificant effects on *Xhc* populations on harvested seeds (Fig. 1, Tables 2 and 3). The results suggest that the efficacy of the various ManKocide® treatments on populations of *Xhc* on the plants had no significant efficacy on the amount of *Xhc* that developed on the harvested seeds. Non-inoculated plots showed an average population of 4.6 Log CFU/g seeds; while plots inoculated with *Xhc* all had more than 6.4 Log CFU/g seed (Fig. 1 and Table 2). All seed lots harvested from plots inoculated with *Xhc* would have had to be hot water-treated.

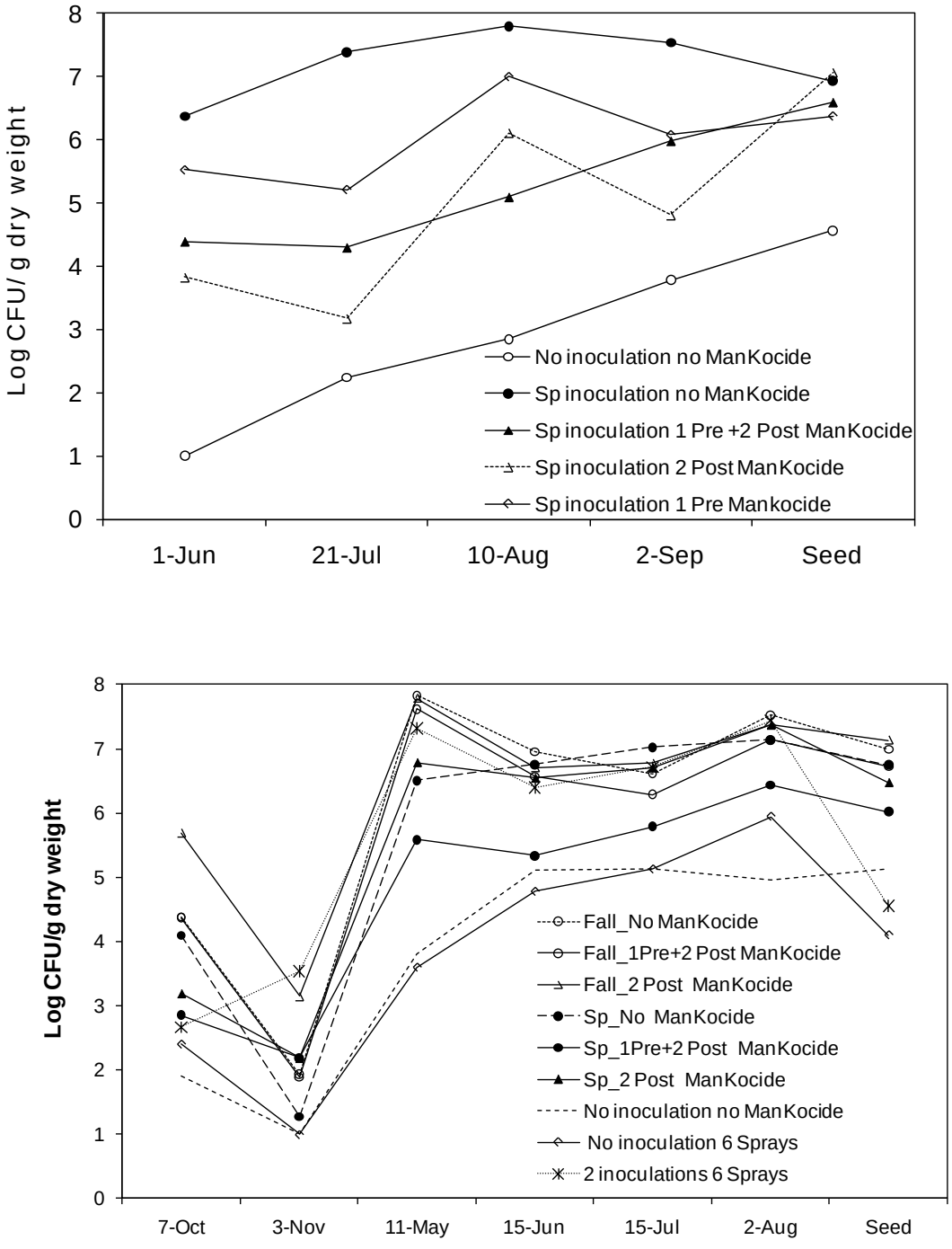

Figure 1. Effects of inoculation with *Xanthomonas hortorum* pv. *carotae* (*Xhc*) and ManKocide treatments on colony-forming units (CFUs) of *Xhc*/g dry carrot tissue or seed determined on different dates in field trials in central Oregon with a seed-to-seed crop (top) and a steckling-to-seed crop (lower), 2008-2009..

Table 1. Population of colony forming units (CFUs) of *Xanthomonas hortorum* pv. *Carotae (Xhc)* on carrot plants and harvested seed in a direct-seeded carrot seed crop trial in central Oregon, 2008-2009.

	Population of *Xhc* on foliage log (CFUs/g dry foliage)*						Log CFUs/ g harvested seeds
Treatment‡	7-Oct	3-Nov	11-May	15-Jun	15-Jul	2-Aug	
No inoculation, no ManKocide	1.9 b	1.0 b	3.8 c	5.1 cd	5.1 b	5.0 b	5.1 bcd
Fall inoculation, no ManKocide	4.4 a	1.9 ab	7.8 a	7.0 a	6.6 a	7.5 a	7.0 ab
Fall inoculation, ManKocide 1 pre-inoc + 2 post-inoc	4.4 ab	1.9 ab	7.6 ab	6.6 ab	6.3 ab	7.1 a	6.7 ab
Fall inoculation, ManKocide 2 post-inoc	5.7 ab	3.2 a	7.8 a	6.7 ab	6.8 a	7.4 a	7.1 a
Spring inoculation, no ManKocide	4.1 ab	1.3 b	6.5 ab	6.8 a	7.0 a	7.1 a	6.8 ab
Spring inoculation, ManKocide 1 Pre-inoc + 2 post-inoc	2.9 ab	2.2 ab	5.6 bc	5.3 bcd	5.8 ab	6.4 ab	6.0 abcd
Spring inoculation, ManKocide 2 post-inoc	3.2 ab	2.2 ab	6.8 ab	6.5 ab	6.7 a	7.4 a	6.5 abc
No inoculation, ManKocide 3 fall + 3 spring	2.4 b	1.0 b	3.6 c	4.8 d	5.1 b	5.9 ab	4.1 d
Fall and spring inoculation, ManKocide 3 fall + 3 spring	2.7 b	3.5 a	7.3 ab	6.4 abc	6.7 a	7.4 a	4.6 cd
LSD (P<0.05)**	Rank	Rank	Rank	Rank	Rank	Rank	Rank

‡ pre-inoc = ManKocide applied to the plants 4 days prior to inoculation of the plants with *Xhc*. post-inoc = ManKocide applied to the plants 3 and 10 days after inoculation of the plants with *Xhc*.

*CFU = colony forming units of *Xhc*/g dry foliage. Data were analyzed on a log scale.

**LSD = Fisher's protected least significant difference following ANOVA. Means with the same letter within a column are not significantly different (*P*<0.05).

Table 2. Colony forming units (CFUs) of *Xanthomonas hortorum* pv. *Carotae (Xhc)* on carrot plants and harvested seed in a steckling seed crop trial in central Oregon, 2009.

	Population of *Xanthomonas hortorum* pv. *carotae* on carrot foliage (log CFUs/g dry foliage)*				
Treatment‡	1-Jun	21-Jul	10-Aug	2-Sep	CFUs/10,000 harvested seeds
No inoculation, no ManKocide	1.0 c	2.2 c	2.8 c	4.1 c	4.6 b
Spring inoculation, no ManKocide	6.4 a	7.4 a	7.8 a	7.5 a	6.9 a
Spring inoculation, ManKocide 1 pre-inoc + 2 post-inoc	4.4 ab	4.3 bc	5.1 b	6.0 ab	6.6 a
Spring inoculation, ManKocide 2 post-inoc	3.8 b	3.2 bc	6.1 ab	5.0 bc	7.1 a
Spring inoculation, ManKocide 1 pre-inoc	5.5 ab	5.2 ab	7.0 ab	6.1 ab	6.4 a
LSD (P<0.05)**	Rank	Rank	Rank	Rank	Rank

‡ pre-inoc = ManKocide applied to the plants 4 days prior to inoculation of the plants with *Xhc*. post-inoc = ManKocide applied to the plants 3 and 10 days after inoculation of the plants with *Xhc*.

*CFU = colony-forming units of *Xhc*/g dry foliage. Data were analyzed on a log scale.

**LSD = Fisher's protected least significant difference following ANOVA. Means with the same letter within a column are not significantly different ($P<0.05$).

Table 3. The results from the analyses of variance ANOV on contrasts on effects of inoculation of plants with *Xanthomonas hortorum* pv. *Carotae (Xhc)* in fall and spring in both a direct-seeded (seed-to-seed) trial and a steckling-to-seed carrot seed crop trial, with applications of ManKocide prior to or following each inoculation, central Oregon, 2008-2009. A probability value of $P<0.05$ indicates a statistically significant difference.

Contrasts	7-Oct	3-Nov	11-May	15-Jun	15-Jul	2-Aug	Harvested seed
Seed-to-seed trial							
No ManKocide applied, non-inoculated vs. inoculated in fall	0.088	0.273	<0.001	0.011	0.037	0.0031	0.060
No ManKocide, non-inoculated vs. inoculated in spring	0.131	0.746	0.015	0.022	0.009	0.011	0.100
Inoculated in fall, ManKocide vs. no ManKocide	0.603	0.425	0.888	0.601	0.888	0.715	0.939
Inoculated in spring, ManKocide vs. no ManKocide	0.389	0.215	0.729	0.182	0.200	0.743	0.548
No inoculation, ManKocide vs. no ManKocide	0.728	1.000	0.843	0.638	0.999	0.241	0.287
Steckling-to-seed trial			1-Jun	21-Jul	10-Aug	2-Sep	Harvested seed
No ManKocide, non-inoculated vs. inoculated in spring			<0.001	<0.001	<0.001	<0.001	0.012
Inoculated in spring, ManKocide vs. no ManKocide			0.048	0.002	0.033	0.024	0.725