Timing Effect of ManKocide® Application on Bacterial Blight on Carrot Seed, 2008

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Abstract

Field plantings were set up to evaluate inoculation timing and ManKocide® application pre- and post-inoculation of carrot foliage with *Xanthomonas hortorum* pv *carotae* (*Xhc*) for suppression of bacterial blight. Application timing was tested on both seed-to-seed carrots and transplanted (steckling) carrot roots. Overall, results show a decline in bacteria populations on plots treated with ManKocide® applications. Seed-to-seed plots were moderately controlled by ManKocide® when applied pre- and post-inoculation. Spring inoculated plots were effectively controlled by two post applications of ManKocide® Transplanted carrots showed a more positive response to pre- and post-inoculation applications than those that received only the post-inoculation applications.

Materials and Methods

Two separate trials were planted under furrow irrigation to test the effectiveness of timing applications of ManKocide® in controlling low concentrations of bacterial blight. A randomized complete block design was used with seven replications of nine treatments for seed-to-seed carrots and seven replications of four treatments for transplanted carrots. Carrots were planted in 30-inch rows in long strips through the field. Strips were separated by a 20-ft unplanted alley. Prior to treatments, carrots were removed by tillage from 30-ft cross-alleys between plots along the strips. Thus, plots were 20 ft wide (8 rows) by 20 ft long, separated by alleys either 20 or 30 ft wide.

A female-only carrot line was planted to prevent this field from cross-pollinating with other fields in the region. Seed-to-seed plots were planted August 14, 2007. Steckling plots were transplanted on April 3, 2008. No bees were installed at pollination, but bees were active on flowers from a nearby hive.

Plots were inoculated with a suspension of 10^2 colony-forming units (CFU)/ml concentration of *Xhc* in 0.0125 M phosphate buffer. Fall-inoculated plots were sprayed on September 21, 2007 and spring-inoculated plots on April 14, 2008. Inoculum was applied using a standard CO_2-pressurized backpack sprayer calibrated to apply suspension in 20 gal/acre. Spray boom is 10 ft in width with 5 nozzles (8002 tips), which allowed coverage over 4 rows at a time. Each plot required two passes to cover all eight rows.

ManKocide® was applied at a rate of 2.5 lb/acre using a commercially designed tractor-mounted spray tank and boom. Applications were targeted for 4 days prior to inoculation, 3 days after inoculation, and 10 days after inoculation. Pre- and post-inoculation applications of fall ManKocide® were performed on September 17 and 24,

and October 1, 2007. Spring ManKocide® applications were applied on April 10, 17, and 24, 2008.

Treatment list for seed-to-seed plots:

1. No inoculation, no ManKocide® application
2. Fall inoculation, no ManKocide® application
3. Fall inoculation, ManKocide®application 1 pre-inoculation + 2 post-inoculation
4. Fall inoculation, ManKocide®application 2 post-inoculation
5. Spring inoculation, no ManKocide®application
6. Spring inoculation, ManKocide®application 1 pre-inoculation + 2 post-inoculation
7. Spring inoculation, ManKocide®application 2 post-inoculation
8. No inoculation, ManKocide®application 3 fall and 3 spring treatments
9. Fall and spring inoculation, ManKocide®application 3 fall and 3 spring treatments

Treatment list for transplanted plots:

1. No inoculation, no ManKocide®application
2. Spring inoculation, no ManKocide® application
3. Spring inoculation, ManKocide®application 1 pre-inoculation + 2 post-inoculation
4. Spring inoculation, ManKocide®application 2 post-inoculation

Monthly sampling dates were October 8 and November 12, 2007 and sampling resumed again on May 5, 2008. Sampling involved collecting foliage from 30 plants per plot, placing foliage into a new plastic bag, and storing each bag refrigerated until plants were assayed within 24 hours of collection. Assay preparations involved chopping the foliage and placing it in a sterile flask with sterile 0.0125 M phosphate buffer. Flasks of foliage and buffer were shaken for 1 hour on a gyratory shaker. The rinsate from each flask was diluted serially up to 10^{-8}. Using sterile technique to avoid contamination, aliquots of each dilution were spread onto XCS agar and incubated at 82°F for 1 week. When plants were small, all the foliage from each composited carrot was sampled. As plants became larger and bolted, plants were subsampled to include a representative amount of foliage, petioles, stems, and umbels.

From September 15 until September 26, umbels were hand clipped from plots as they matured. One hundred umbels representing a typical harvest range (mostly primary and secondary) were collected and bagged and allowed to further air dry. Hands and tools were disinfected following collection from each plot. After several additional weeks air drying, seed was hand rubbed from each umbel per plot. Seed was deburred, and then passed through screens by hand, using standard research equipment. This procedure simulated the commercial combine-deburring seed-cleaning process. All tools, equipment, and hands were disinfected between each plot sample at each step of the process. Each seed sample was soaked overnight at 4°C in 100 ml of saline (0.85 percent NaCl). Two drops of Tween® 20, a nonionic detergent, were then added to each

flask, which was placed on a rotary shaker for 5 min. A dilution series of the wash was plated onto XCS agar medium. Colony-forming units are expressed based on a 10,000 seed sample.

Results and Discussion

Seed-to-seed field

Inoculation was successful as all seven inoculated but non- ManKocide® treated plots tested positive. Foliage symptoms were not apparent until almost 3 months after first inoculation. Symptom severity was not measured due to size of plots and removal of plants during sampling. A low level of disease was not detected on non-inoculated plots until June and then was limited to one or two isolated incidents. Fall-inoculated plants not treated with ManKocide® and those treated with ManKocide® post-inoculation showed a higher final population than those treated pre- and post-inoculation (Table 1). Spring-inoculated plants not treated with ManKocide® and plots treated with ManKocide® post-inoculation showed a higher final population than those treated pre- and post-inoculation. Overall, on average, those plots treated with ManKocide® developed populations of bacteria on seeds under thresholds needed to avoid hot water treatments. Plots inoculated in both fall and spring showed the highest seed bacteria populations of the trial. Bacterial levels on seed were not statistically significant but plots treated with ManKocide® did show a tendency for bacteria levels to be below thresholds.

Steckling field

The initial steckling lot was tested for *Xhc*, resulting in 3 out of 20 roots testing positive for bacteria. The non-inoculated, no ManKocide® check plots did periodically show bacteria populations, which may be due to the infested root stock. Inoculation was successful as all seven inoculated non- ManKocide® treated plots tested positive. End of season bacteria populations were highest on plots inoculated but not treated with ManKocide® and those treated with two post application of ManKocide® (Table 2). Bacteria populations on harvested seed were below thresholds for both treatments using ManKocide® although the two post- ManKocide® applications showed a lower population than the pre- and post-inoculation treatment.

Results favor the importance of using ManKocide® as an effective preventative control of bacterial blight. A repeat trial is scheduled for 2008-2009 to verify relative effectiveness. Humidity was not measured but will be a parameter monitored in the 2008-2009 trial.

Table 1. Population of colony-forming units (CFU) of bacterial blight on seed-to-seed carrot plots.

Treatment	Population of bacterial blight on foliage LOG (CFUs/g dry foliage)* 15 Oct	12 Nov	5 May	2 Jun	14 Jul	11 Aug	CFUs/10,000 seeds Harvest seed
No inoculation, no ManKocide®	1.0 c	1.0 c	1.0 c	2.3 cde	1.0 d	1.9 e	2.2
Fall inoculation, no ManKocide®	4.1 a	4.8 a	5.2 a	4.6 a	6.1 a	7.6 a	5.2
Fall inoculation, ManKocide®1 pre-inoc + 2 post-inoc	1.3 bc	1.7 b	2.2 bcd	3.0 bc	4.6 ab	5.6 bc	5.1
Fall inoculation, ManKocide®2 post-inoc	1.9 b	1.0	3.7 ab	2.7 bcd	3.2 bc	6.5 ab	4.5
Spring inoculation, ManKocide®	1.0 c	1.0 c	3.1 ab	3.8 ab	5.6 a	6.3 ab	5.6
Spring inoculation, ManKocide®1 pre-inoc + 2 post-inoc	1.0 c	1.0 c	1.0 d	1.8 cde	1.9 cd	4.2 cd	4.0
Spring inoculation, ManKocide®2 post-inoc	1.0 c	1.0 c	1.0 d	1.9 cde	3.6 bc	2.0 e	4.6
No inoculation, ManKocide®3 fall + 3 spring	1.0 c	1.0 c	1.6 cd	1.0 e	2.7 bcd	3.6 de	3.1
Fall and spring inoculation, ManKocide®3 fall + 3 spring	1.0 c	1.0 c	1.1 d	1.2 de	3.5 bc	4.8 bcd	5.8
LSD ($P < 0.001$)**	0.8	0.7	1.6	1.5	1.9	1.9	*NS*

*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 ($P < 0.005$).

Table 2. Population of colony-forming units (CFU) of bacterial blight on transplanted carrot plots.

Treatment	Population of bacterial blight on foliage LOG (CFUs/g dry foliage)* 28 May	24 Jun	5 Aug	3 Sep	CFUs/10,000 seeds Harvested seed
No inoculation, no ManKocide®	1.0 b	1.8 b	2.0 b	2.5 c	4.1 ab
Spring inoculation, no ManKocide®	4.7 a	5.9 a	6.8 a	7.2 a	6.5 a
Spring inoculation ManKocide®1 pre-inoc + 2 post-inoc	3.3 a	2.9 b	2.6 b	4.1 bc	5.0 ab
Spring inoculation, ManKocide® 2 post-inoc	4.5 a	3.5 b	3.3 b	5.6 ab	3.6 b
LSD ($P < 0.001$)**	2.0	2.3	2.0	2.2	2.8

*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 ($P < 0.005$).