Evaluating Actigard® for Controlling Xanthomonas Blight in Carrot Seed Crops

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Introduction

Bacterial blight caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*), is a major disease in fresh carrots. The pathogen is commonly seedborne, and contaminated seed is an important inoculum source for development of bacterial blight in the fresh crop production fields.

In carrot seed fields in central Oregon, bacterial blight generally is mild and sporadic, but even in the absence of disease symptoms in fields, the pathogen can be abundant on seeds harvested in this region. Seed lots with moderate to high levels of contamination must be treated with hot water to reduce the infestation level. Hot water treatment is expensive and can reduce the germination rate and vigor of seeds.

Several management recommendations have been developed to suppress (partially) *Xhc* contamination of carrot seed, including 2- to 4-year crop rotations to non-hosts, planting pathogen-free seed, incorporating residues into the soil promptly after harvest, avoiding overhead irrigation that promotes pathogen dispersal and host colonization, and applications of copper bactericides. Although copper-containing products are regularly applied to central Oregon carrot seed fields to prevent pathogen contamination of seed, previous studies have shown that the efficacy of these treatment are, at best, marginal (Simmons et al. 2009, 2010).

Systemic acquired resistance (SAR) is an induced defense mechanism that confers long-lasting protection to host plants against a broad spectrum of pathogens. SAR is characterized by the accumulation of the signal molecule salicylic acid (SA) and is associated with increasing expression of host genes that contribute to resistance. SAR can be triggered in plants by treatment with elicitor compounds. One elicitor of SAR is acibenzolar-S-methyl (ASM), which is marketed as Actigard® (Syngenta Crop Protection, Inc., Greensboro, NC). ASM has demonstrated efficacy in inducing SAR in numerous hosts, preventing or reducing diseases caused by a variety of bacterial, fungal, and viral pathogens. A recent study in citrus showed long lasting induction of SAR with soil application of Actigard. These soil treatments resulted in significant and economic control of citrus canker caused by *Xanthomonas citri* subsp. *citri*, which is very similar to *Xhc* in biology.

The objective of this study was to evaluate soil treatments of Actigard for controlling *Xhc* in carrot seed crops.

Materials and Methods

Field Trial

A field trial was conducted at the Central Oregon Agricultural Research Center in Madras, Oregon. The following six treatments were arranged according to a randomized complete block design with six replications:

1. Untreated check, no Actigard or ManKocide® (fungicide/bactericide) applied;
2. Actigard at 2 oz/acre on June 18, July 7, and August 4 (total 6 oz/acre);
3. Actigard at 4 oz/acre on June 18, July 7, and August 4 (total 12 oz/acre);
4. Actigard at 4 oz/acre on June 18 and July 23 (total 8 oz/acre);
5. Actigard at 8 oz/acre on June 18 and July 23 (total 16 oz/acre);
6. ManKocide applied at 2.5 lb/acre twice after new growth of stecklings and at bolt.

Each plot consisted of an area 20 ft by 20 ft planted with female carrot stecklings, 2 plants/ft on April 27, 2010. Plots were separated by 20-ft alleys between plots on all sides. Drip tape was installed on May 17 with an 8-row installer. Drip irrigation was designed using five layflat lines, each line representing one Actigard treatment. From each layflat hose, a compression adapter connected poly tubing that carried the water to each plot of eight rows of drip tape. The system was intended to allow one treatment (six replications) to be irrigated at the same time. Pressure was checked to ensure plots at the end of the irrigation line were getting the same amount of water as those nearest the line. Actigard was injected to the system using a Dosmatic™ Advantage A30 in-line injector.

First drip irrigation water occurred on June 4, 2010. The field was inoculated uniformly on June 25, 2010 by spraying the plots with an *Xhc* suspension (10^2 cells/ml) at 20 gal/acre. Because *Xhc* was not detected in samples taken 1 week post inoculation, plots were inoculated a second time on July 13, 2010 (10^4 *Xhc* cells/ml and 40 gal water/acre). Plots were sampled monthly starting from 1 week post inoculation. Carrot leaves and umbels were randomly collected from 20 plants and combined for each plot. The sample tissues were chopped into small pieces, mixed well and a subsample of about 12 g fresh weight was drawn for assay. The subsamples for assay were soaked in 150 or 200 ml phosphate buffer for 1 hour on a gyratory shaker. A dilution series of 10^0, 10^{-1}, 10^{-3}, and 10^{-5} of the rinsate was prepared, and each dilution spread onto an XCS agar plate at 0.1 ml per 90-mm-diameter Petri dish. The plates were incubated at 82ºF in the dark for 1 week, and then the numbers of *Xhc* colonies were counted. The plant tissue was recovered immediately after rinsing, dried at 55ºC, and weighed to determine the dry weight for each subsample. The number of colony-forming units (CFUs) per g of dry weight was calculated based on the dilution plated, the number of colonies on the plate, and the dry weight of the corresponding subsample. At maturation, carrot umbels were randomly harvested from plants, air dried in clean paper bags at room temperature for 3-4 weeks, and then seeds were hand-rubbed off, deburred, and passed through screens using standard research equipment mimicking the commercial combine-deburring and seed-cleaning processes. All tools, equipment, and hands were disinfected between samples at each step of the process.

Seed samples were further dried; a subsample of approximately 20 g of seeds was drawn from each sample and assayed for *Xhc* population. The seed sample was soaked in a flask with 200 ml saline (0.85% NaCl) overnight at 4ºC. Two drops of Tween® were then added to each flask and mixed by shaking on a rotary shaker for 5 minutes. A dilution series of the rinsate was spread onto XCS agar plates and CFUs counted after a week of incubation at 82ºF as described for leaf and umbel samples. For each sample, total CFUs was divided by dry weight of the sample and then log-transformed to log CFU/g dry weight.

Greenhouse Experiments

Two experiments were conducted one after the other in the greenhouse in 2010. The second trial was planted after the first study was removed from the greenhouse. The carrot cultivar seeds were planted in standard potting soil, and thinned to 1 seedling per pot at the 2-true-leaf stage. At the 4-leaf stage, carrot seedlings were subjected to the following 13 treatments:

1. Actigard soil drench at 5 mg/pot, and inoculated with *Xhc* 1 week later;
2. Actigard soil drench at 10 mg/pot, and inoculated with *Xhc* 1 week later;
3. Actigard soil drench at 15 mg/pot, and inoculated with *Xhc* 1 week later;
4. no Actigard treatment, inoculated 1 week later;
5. Actigard soil drench at 5 mg/pot, and inoculated with *Xhc* 3 weeks later;

6. Actigard soil drench at 10 mg/pot, and inoculated with *Xhc* 3 weeks later;
7. Actigard soil drench at 15 mg/pot, and inoculated with *Xhc* 3 weeks later;
8. no Actigard treatment, inoculated 3 weeks later;
9. Actigard soil drench at 5 mg/pot, and inoculated with *Xhc* 6 weeks later;
10. Actigard soil drench at 10 mg/pot, and inoculated with *Xhc* 6 weeks later;
11. Actigard soil drenching at 15 mg/pot, and inoculated with *Xhc* 6 weeks later;
12. no Actigard treatment, inoculated 6 weeks later;
13. untreated check, no Actigard treatment, no inoculation.

Eight replicates (pots) were included for each treatment. The pots were arranged in a complete random design. Soil drenching included suspending the amount of Actigard in 100 ml water and drenching at the 4-leaf stage. Inoculation of carrot seedlings was done by spraying *Xhc* suspension at 10^4 cells/ml. Six weeks after inoculation, the bacterial population on leaves was assayed. The leaves were chopped into small pieces and soaked in 20 ml phosphate buffer for 1 hour on a gyratory shaker. A dilution series of 10^0, 10^{-1}, 10^{-3}, and 10^{-5} of the rinsate was prepared and spread onto an XCS agar plate at 0.1 ml per 90-mm-diameter Petri dish. *Xhc* colonies were counted after incubation, and log CFU per g dry weight was determined as described in the field trial.

Results and Discussion

Field Trial

The spring of 2010 was wet and cold. The transplanting of carrot stecklings and subsequent harvest was postponed compared with most years. No obvious abnormal growth was observed for plants treated with Actigard at any of rates tested in this experiment. *Xhc* was not detected on the first three sampling dates June 19, and July 1, and 19 during this trial, although *Xhc* was common to central Oregon. The bacterial population on leaves and umbels remained relatively low on August 16 and September 13 (upper Figure 1). *Xhc* population on seeds ranged from undetectable to 6.3×10^6 CFUs per g dry seed, and mean log CFUs for the 6 treatments ranged from 4.7 to 6.1 (lower Fig. 1), lower than previous years (Simmons et al. 2009, 2010). The growth of carrot plants was uneven, and plants were smaller in one side of the trial field. The significant effects of block on *Xhc* populations were also detected in analysis of variance (Table 1). When data from different sampling dates were analyzed separately, effects of Actigard treatments were not significant on *Xhc* populations on leaves and umbels (Table 2). However, 2 or 3 applications of Actigard at 4 oz/acre significantly reduced the *Xhc* population on seeds whereas 3 applications of Actigard at 2 oz/acre and 2 applications of Actigard at 8 oz/acre did not affect Xhc populations significantly (Table 2, and lower Figure 1). The *Xhc* populations between sampling dates showed very low correlation (data not shown).

Greenhouse Experiment

Due to the fact that the two greenhouse experiments were conducted at different times, the conditions in the greenhouse changed from the first experiment to the second. The results exhibited significant effects of the experiment, and the interactions of rate by experiment, rate by week by experiment, and week by experiment were all significant (Table 3). Therefore, the data from the two experiments were analyzed separately. Significant effects of Actigard rate was detected in the second experiment whereas significant effects of Actigard on *Xhc* populations were not detected due to large differences between inoculation times (Table 4 and Figure 2). The difference among time lapses between treatment and inoculation was significant in both experiments, but the effects varied between experiments. The *Xhc* population was highest when

plants were inoculated 1 week after Actigard treatments in the first experiment, but in the second experiment it was highest when plants were inoculated 6 weeks after Actigard treatments (Figure 2, mean comparison was not shown). When correlation between *Xhc* population levels and Actigard dose in drench treatment was analyzed separately according to experiment and inoculation time, the dose effects were significant for the plants inoculated 6 weeks after drench in the first experiment, and for plants inoculated 3 weeks after drench in the second experiment (Table 5). In both cases, the *Xhc* population was significantly lower as the dose of Actigard increased (Table 5 and Figure 2).

The inconsistent results between two greenhouse experiments suggested variable environmental conditions (including temperature, light, humidity, etc.) might have significantly contributed to the difference in *Xhc* populations between experiments. These variations might have also contributed to the difference among inoculation times because the conditions in the greenhouse varied at different inoculation times. In order to determine how long the effects of Actigard treatments can last, and when the effects were strongest, more studies are needed with better control of these conditions over time.

The results from the field trial were not conclusive. Results suggested that although Actigard treatments did not reduce *Xhc* population on carrot leaves and umbels, they might reduce *Xhc* population on carrot seeds. More studies are needed to confirm these results and to investigate why Actigard drench may reduce the seed bacterial population but has no significant effects on leaf and umbel population. It is also worthy to evaluate the possibility of controlling *Xhc* population on carrot seeds through integration of Actigard drench with other practices, such as foliage spray of Actigard and copper bactericides.

Table 1. Analysis of variance on log colony-forming units of *Xhc* in leaf and umbel samples collected on August 16 and September 13 and on seeds at harvest from plots subjected to different treatments in a field trial at the Central Oregon Agricultural Research Center, Madras, OR.

Source	DF	Type III SS	Mean square	F value	Pr > F
Treatment[a]	5	13.2	2.6	1.71	0.141
Sample date	2	6.5	3.2	2.09	0.130
Treatment × sample date	10	31.4	3.1	2.04	0.039
Block	5	18.8	3.8	2.44	0.041

[a]: Six treatments were included: 1) untreated check; 2) 3 Actigard applications at 2 oz/acre; 3) 3 Actigard applications at 4 oz/acre; 4) 2 Actigard applications at 4 oz/acre; 5) 2 Actigard applications at 8 oz/acre; and 6) 2 ManKocide applications at 2.5 lb/acre.

Table 2. Mean comparison for log (colony-forming units per g dry weight) of *Xhc* in leaf and umbel samples collected on August 16 and September 13 and on seeds at harvest from plots subjected to different treatments in a field trial at the Central Oregon Agricultural Research Center, Madras, OR.

Treatment[a]	Sample Aug 16		Sample Sep 13		Seed at harvest	
1	4.4	a	4.5	bc	6.1	a
2	4.7	a	4.6	bc	5.4	ab
3	4.9	a	5.1	abc	4.7	b
4	4.5	a	6.5	a	4.8	b
5	4.7	a	6.0	ab	5.2	ab
6	4.4	a	3.5	c	4.8	b

[a]: Six treatments were included: 1) untreated check; 2) 3 Actigard applications at 2 oz/acre; 3) 3 Actigard applications at 4 oz/acre; 4) 2 Actigard applications at 4 oz/acre; 5) 2 Actigard applications at 8 oz/acre; and 6) 2 ManKocide applications at 2.5 lb/acre.

Table 3. Analysis of variance on log colony-forming units of *Xhc* on carrot seedlings drenched with 0, 5, 10, and 15 mg Actigard per pot (plant) and inoculated with *Xhc* 1, 3, and 6 weeks after drench in 2 greenhouse experiments, Central Oregon Agricultural Research Center, Madras, OR.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Rate (of Actigard drench)	3	32.62	10.87	3.63	0.0142
Week (post drench)	2	43.42	21.71	7.25	0.0010
Rate × week	6	21.64	3.61	1.20	0.3066
Experiment	1	63.37	63.37	21.15	<.0001
Rate × Experiment	3	30.05	10.02	3.34	0.0206
Week × Experiment	2	90.31	45.16	15.07	<.0001
Rate × Week × Experiment	6	47.13	7.86	2.62	0.0187

Table 4. Analysis of variance on log CFU of *Xhc* on carrot seedlings drenched with 0, 5, 10, and 15 mg Actigard per pot (plant) and inoculated with *Xhc* 1, 3, and 6 weeks after drench in two greenhouse experiments, Central Oregon Agricultural Research Center, Madras, OR.

Source	DF	Type III SS	Mean square	F value	Pr > F
Experiment 1					
Rate (of Actigard drench)	3	5.90	1.97	0.71	0.5510
Week (post drench)	2	37.71	18.85	6.77	0.0019
Rate × week	6	33.45	5.57	2.00	0.0745
Experiment 2					
Rate	3	56.77	18.92	5.90	0.0011
Week	2	96.02	48.01	14.97	<.0001
Rate × week	6	35.33	5.89	1.84	0.1017

Table 5. Correlation coefficients between *Xhc* population on carrot seedlings drenched with Actigard and the dose of Actigard drench, Central Oregon Agricultural Research Center, Madras. OR.

	Inoculation time (weeks post drench)		
	1	3	6
Experiment 1	-0.55	+0.56	**-0.99****
Experiment 2	-0.51	**-0.95****	-0.10

**: the correlation coefficients are significant at $P < 0.05$.

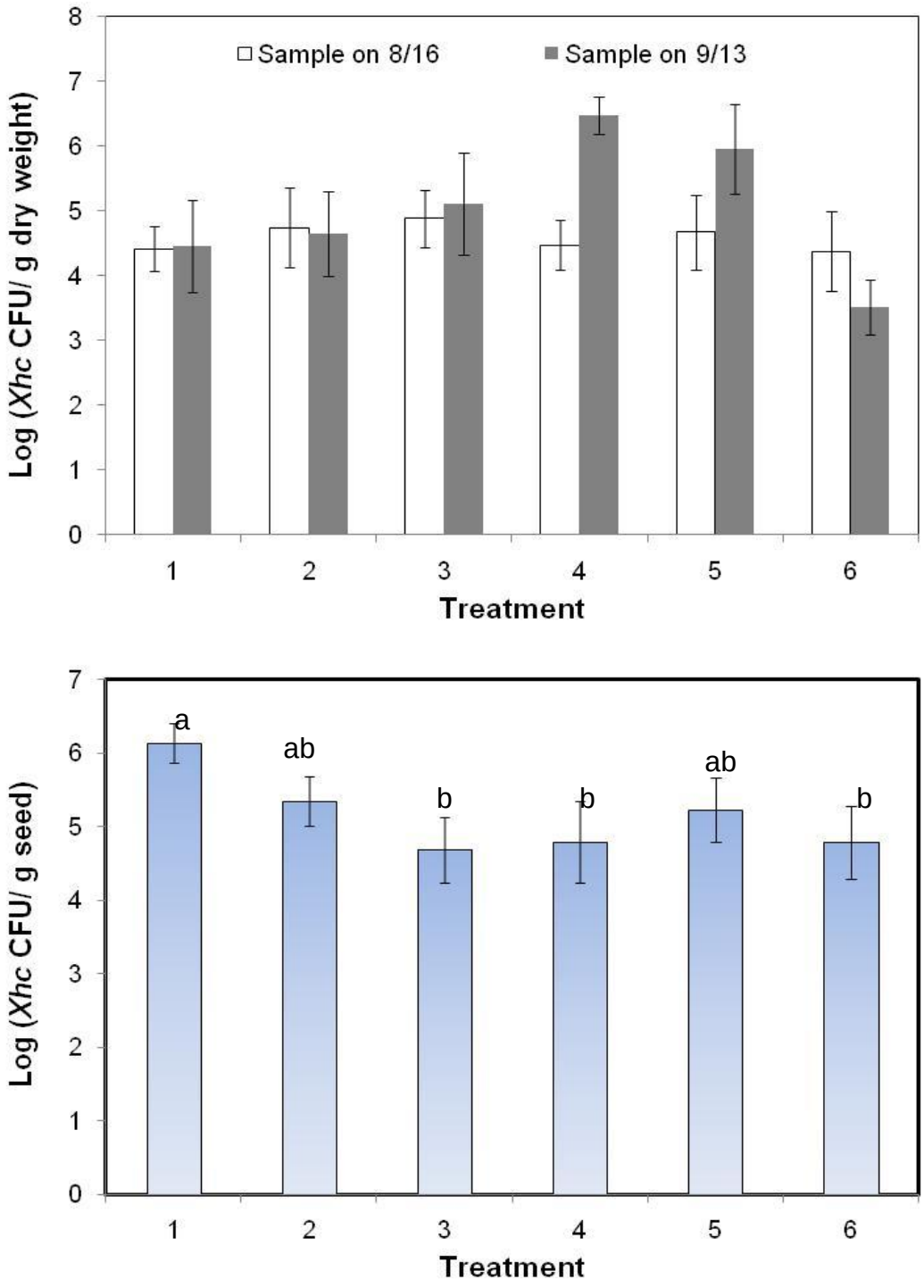

Figure 1. *Xhc* population on carrot plants (upper) and seeds (lower) from plots subjected to different treatments: 1) untreated check; 2) 3 Actigard applications at 2 oz/acre; 3) 3 Actigard applications at 4 oz/acre; 4) 2 Actigard applications at 4 oz/acre; 5) 2 Actigard applications at 8 oz/acre; and 6) 2 ManKocide applications at 2.5 lb/acre. The data are means of 6 replicates and the vertical bars are standard errors of the means. Central Oregon Agricultural Research Center, Madras, OR.

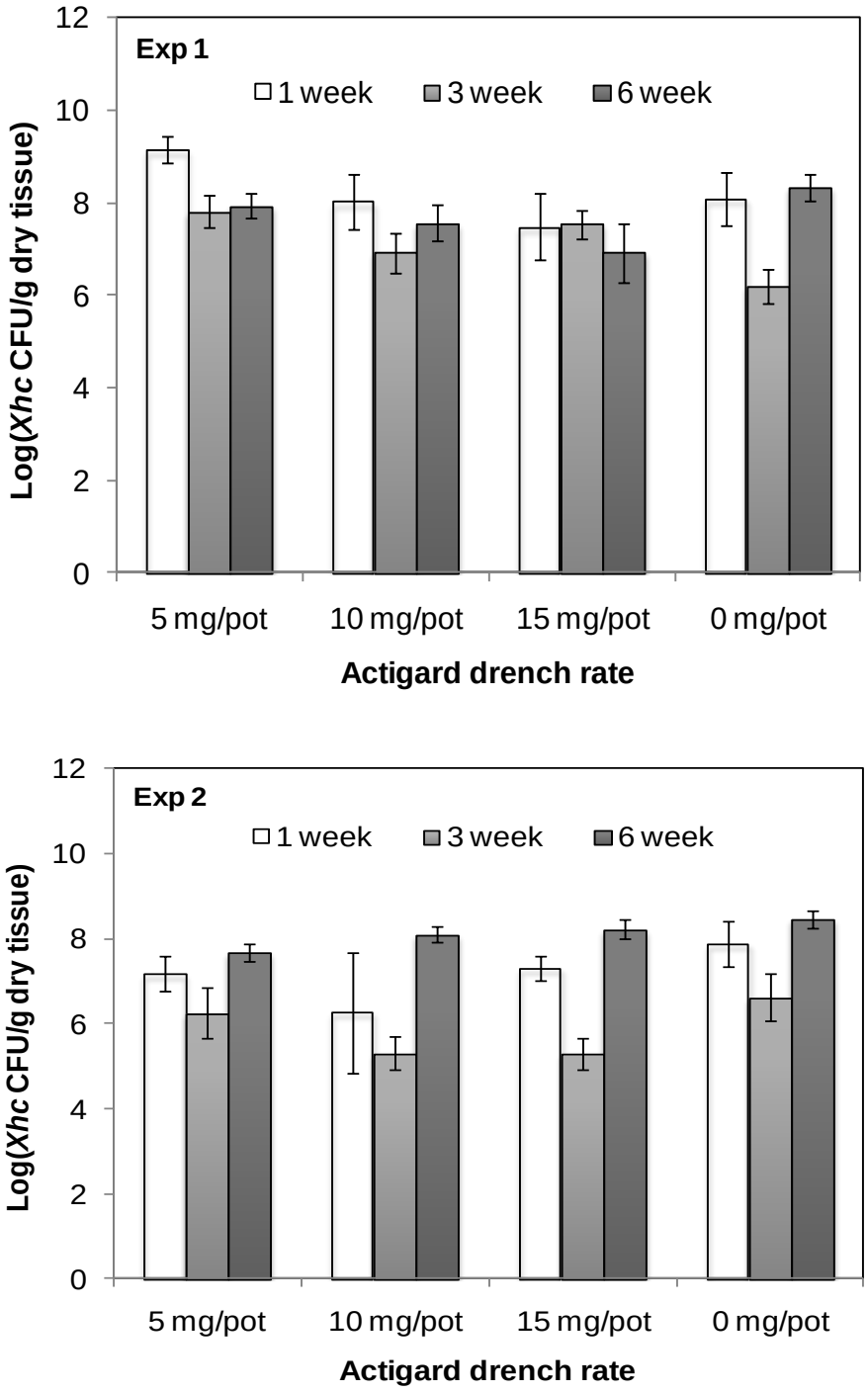

Figure 2. *Xhc* population on carrot seedlings drenched with 0, 5, 10, and 15 mg Actigard per pot and inoculated with 100 ml *Xhc* suspension at10^4 cells/ml 1, 3, and 6 weeks after drench in 2 greenhouse experiments, Central Oregon Agricultural Research Center, Madras, OR.

References

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