Evaluate Actigard and Bactericides for Suppression of Xanthomonas in Carrot Seed Crops

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Introduction

Bacterial blight caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*) is a major disease in commercial carrot production. Because seed contaminated with *Xhc* initiates disease development in commercial carrot production, *Xhc* is the most important concern to the carrot seed industry in the Pacific Northwest.

Copper-containing products such as ManKocide® are applied regularly to carrot seed crops in the Pacific Northwest in an attempt to prevent contamination of seed by *Xhc*, but the effect of this treatment on bacterial populations on leaves is generally short-term and its efficacy in reducing bacterial populations on seeds is typically poor to fair. Other alternative chemical strategies, including foliar applications of Actigard® have also shown limited efficacy. Seed lots produced in the region are often hot water-treated to reduce *Xhc* contamination to acceptable levels. However, hot water treatment of seed is expensive owing to the time and precision equipment involved and the infrastructure needed to treat the seed safely. In addition, hot water treatment can have the undesirable side effect of reducing seed germination and seedling vigor.

A study on citrus bacterial canker suggested that root drench with Actigard®, an inducer of systemic acquired resistance (SAR), offered longer and better protection against bacterial canker than foliar treatment. Since drip irrigation has been widely adopted in the semi-arid production areas of central Oregon and central Washington, it provides an ideal method by which to introduce SAR inducers to carrot plants via the roots**.** It is interesting to investigate whether application of SAR inducers via the drip irrigation system can provide protection of carrot leaves, flowers and umbels against *Xhc*, and ultimately reduce the pathogen on the harvested seeds.

The results from of 2010 field trial and greenhouse experiments were not conclusive, but suggested insignificant effects of Actigard® treatment. Objectives in 2011 were 1) to determine the effects of dose and timing of Actigard® drenches on populations of *Xhc* on carrot seed plants grown from stecklings; 2) to evaluate Actigard® introduced via drip irrigation for suppression of *Xhc* in carrot seed crops; and 3) to evaluate combinations of bactericides and Actigard® for suppression of *Xhc* in carrot seed crops.

Materials and Methods

Field trials. Two field trials were conducted at the Central Oregon Agricultural Research Center (COARC) in Madras, Oregon. The plots in the first trial were 20 ft × 20 ft, and arranged according to a randomized complete block design with 6 replicates (see attached plot map 1). Carrot roots were transplanted on April 13 at across-row and in-row spacings, 30 and 9 inches, respectively. Seven treatments were included in the trial. Although natural inoculum of *Xhc* is ubiquitous at COARC, each plot was split into two halves, one half was inoculated with

suspension of laboratory produced *Xhc* (10^4 cells/ml, 40 gal/acre) on June 16th while no inoculation was completed for the other half.

1) No treatment control;
2) Actigard® via drip 4 oz/a on 7/5;
3) Actigard® via drip 4 oz/a on 7/5 and 8/2;
4) Actigard® via drip 4 oz/a on 6/8, 7/5 and 8/2;
5) Phyton 27® on 6/10 and 6/25;
6) Phyton 27® on 6/10 and 6/25, and Actigard® 4 oz/a via drip on 7/5 & 8/2;
7) ManKocide® on 6/10 and 6/25.

Plots in the second trial consisted of 4 carrot plants in a row (across-row and in-row spacings were 30 and 9 inches, respectively. Carrot roots same as used in the 1st trial were transplanted on April 25th. All plants were inoculated with Xhc suspension (10^4 cells/ml, 10 ml/plant) on June 16th. The following 12 treatments were included in the second trial and arranged according to a randomized complete block design with 7 replicates.

1) Actigard® drench 3 times (6/8,7/5, 8/2) 10 mg/plant;
2) Actigard® drench 3 times (6/8,7/5, 8/2) 20 mg/plant;
3) Actigard® drench 3 times (6/8,7/5, 8/2) 40 mg/plant;
4) Actigard® drench 2 times (7/5, 8/2) 10 mg/plant;
5) Actigard® drench 2 times (7/5, 8/2) 20 mg/plant;
6) Actigard® drench 2 times (7/5, 8/2) 40 mg/plant;
7) Phyton 27® twice (6/10, 6/29)+Actigard® drench 2 times (7/5, 8/2) 20 mg/plant;
8) Uptake® twice (6/10, 6/29) + Actigard® drench 2 times (7/5, 8/2) 20 mg/plant;
9) ManKocide® twice on 6/10 and 6/25;
10) Phyton 27® twice on 6/10 and 6/25;
11) Uptake® twice on 6/10 and 6/25;
12) No treatment controls

ManKocide® and Phyton 27® were sprayed with a backpack sprayer at 2.5 lbs/acre and 5 oz/acre, respectively with 40 gallon water per acre in the large plot trial. ManKocide®, Phyton 27® and Uptake® were sprayed with a hand sprayer at a concentration of 1.50%, 0. 38% and 0.80%, respectively. Spray volume for the first spray on June 16th and the second spray on June 25th were 6 ml and 9 ml per plant, respectively. Once (miniplot) or twice (large-plot) a month, 10 leaves and umbels were collected from each plot or subplot (as for the large plot trial) and washed with phosphate buffer. A series of dilutions of washing liquids were plated on XCS, a semiselective medium for *Xhc.* Number of *Xhc* colonies on each plate was numerated after incubation at 82°F for a week. The fresh and dry weights of samples were also determined for calculating CFU/fresh and dry weight.

At harvest, 100 and 25 umbels were randomly collected from each subplot or plot, respectively, in large plot and miniplot trials. Seeds were assayed before deburring and after deburring and cleaning in laboratory for *Xhc* population (as described prevously). CFU per 10g dry seed weight were determined.

Greenhouse studies. Two experiments were conducted in a greenhouse in 2011. In the first experiment, carrot seedlings were planted in 3-inch-diameter foam cups. At the growth stage of 3rd true leaf, the following treatments were applied: 1) check—sprayed with water; 2) ManKocide®; 3) Phyton 27®;4) Phyton 016B®; 5) Uptake®. Four days after treatment, the seedlings were inoculated with laboratory cultured *Xhc*. One to three loops of bacterial colonies were transferred from a fresh culture of *Xhc* on XCS into 250-cc flasks with about 70 ml 523 liquid medium. The flasks were then shaken 2 rpm for 18 hours at room temperature. The suspension was harvested and adjusted to 10^6 cells/ml using a turbidity meter and then diluted to 10^4 cell/ml for spaying the carrot seedlings. An uninoculated check was also included as checks. The seedlings were than grown in a greenhouse for 4-6 weeks. Leaves were collected and assayed for *Xhc* population in the laboratory as described above. In the second experiments, the carrot seedlings were not treated with chemicals, and inoculated with different *Xhc* strains, two rifampicin resistant mutants and one wild type strain in the same way as for chemical screen.

Results and Discussion

In both field trials, Xanthomonas blight symptoms were not observed until the end of the season. Carrot plants remained healthy and grew normally with low level of powdery mildew developing at the end of the season. There was no significant difference in powdery mildew incidence and severity between treatments or between replicates (data not shown). In the small plot trial, the *Xhc* population remained low on plant tissues throughout the season except on seeds tested after harvest. It was lower than 10^3 CFU/g dry tissue for leaf and umbel samples in all the monthly tests from June 15 (before the inoculation) to September 23 (Fig. 1). Despite of the very low *Xhc* population in plant tissues, the population of *Xhc* on seeds was beyond an acceptable threshold and reached as high as more than 10^7 CFU/g dry seeds. The difference in *Xhc* levels was insignificant between different chemical treatments and between the different doses of Actigard (Fig. 1).

Similarly, the population of *Xhc* remained low, lower than 10^4 CFU/g dry tissue on leaves and umbels throughout the growing season in both inoculated and non-inoculated halves regardless of the treatments (Fig. 2). The *Xhc* levels in leaf and umbel samples exhibited no significant difference between treatments, or between non-inoculated and inoculated halves of plots. However, *Xhc* levels on seeds were significantly higher in the halves inoculated with *Xhc* than in the halves without inoculation. In general, *Xhc* levels were higher on deburred seeds than on seeds cleaned only preliminarily by hands, and the difference between inoculated and non-inoculated halves was greater for deburred seeds than for the undeburred seeds.

Results from the greenhouse experiments followed the similar trend to those in the field trials. No significant difference was detected among the *Xhc* population levels on plants sprayed with ManKocide®, Phyton 016B®, Phyton 27® and Uptake® (Fig. 3).

Results from the greenhouse experiments also demonstrated that the two rifampicin resistant *Xhc* mutant strains were comparable to their wild type parent in aggressiveness (Fig. 4). This suggested that the two rifampicin resistant strains can be used in future greenhouse and field trials, allowing the use of less expensive rifampicin amended medium for quantification of *Xhc* population levels.

It was interesting to note that the Xhc population on deburred seeds was higher than on seeds before deburring. The possible explanation to this includes 1) the *Xhc* population on seed increased during the process; 2) the Xhc population level was higher on seeds than on the burs; 3) the deburring and the subsequent cleaning process reduced the inhibitors of *Xhc* so that more *Xhc* CFU on XCS. A separate study was conducted in the laboratory during 2012. Five grams of carrot seed was washed 6 times with 100 ml sterilized 0.85% NaCl and then once again after grinding. The washing liquids were all plated on XCS plates and the CFU of *Xhc* were enumerated as described above. The results revealed that for the majority of the samples tested, levels of *Xhc* population changed very little in the first 5 to 6 successive washings (data not shown). However, the level of *Xhc* population inside the seeds (which assumedly can be detected after grinding) was much higher than the level of *Xhc population* on the seed surface (which assumedly can be washed into the liquid before grinding) (Table 1).

Results from field trials and laboratory experiments demonstrated that none of the treatments tested significantly reduced/affected the *Xhc* population levels on carrot plants or on harvest seeds. The seed assay on selected seed samples from the trials revealed that the majority of seedborne *Xhc* are located inside carrot seeds, suggesting that internal reproduction of *Xhc* may be critical to its seedborne nature. This may explain why no effects of those chemical treatments were detected because none of them are able to reach inside plants/seeds. As for Actigard®, since its effects from induced resistance is to defend plants from infection/penetration instead of reducing the reproduction of pathogens inside plant tissues, it is not surprising to see no effects from this treatment on *Xhc* population inside plant tissues/seeds. If this can be proved true, additional strategies for suppression the seedborne *Xhc* population are needed.

Table 1. Levels of Xhc in washing liquids before and after grinding the seed samples from the large plot field trial in 2012.

Seed	Xhc		After grinding /
Samples	Before grinding[1]	After grinding	before grinding
	------(CFU/g seed)------		
22E	405000	4775000	11.80
18E	710000	19000000	26.80
38E	40000	5450000	136.30
41W	15000	350000	23.30
3W	4000	11750	2.90
3E	927500	12700000	13.70
16W	890000	8375000	9.40
9E	3000	500	0.20
Average			28.00
Regression slope[2]			15.02

[1]: The CFU/g seed data is from the last washing, the 6th washing liquid, except for the sample 3W, for which no Xhc was detected in the 6th washing liquid, the data shown was from the 5th washing liquid.

[2]: the slope was estimated by regression of CFU/g after grinding against the corresponding CFU/g before grinding

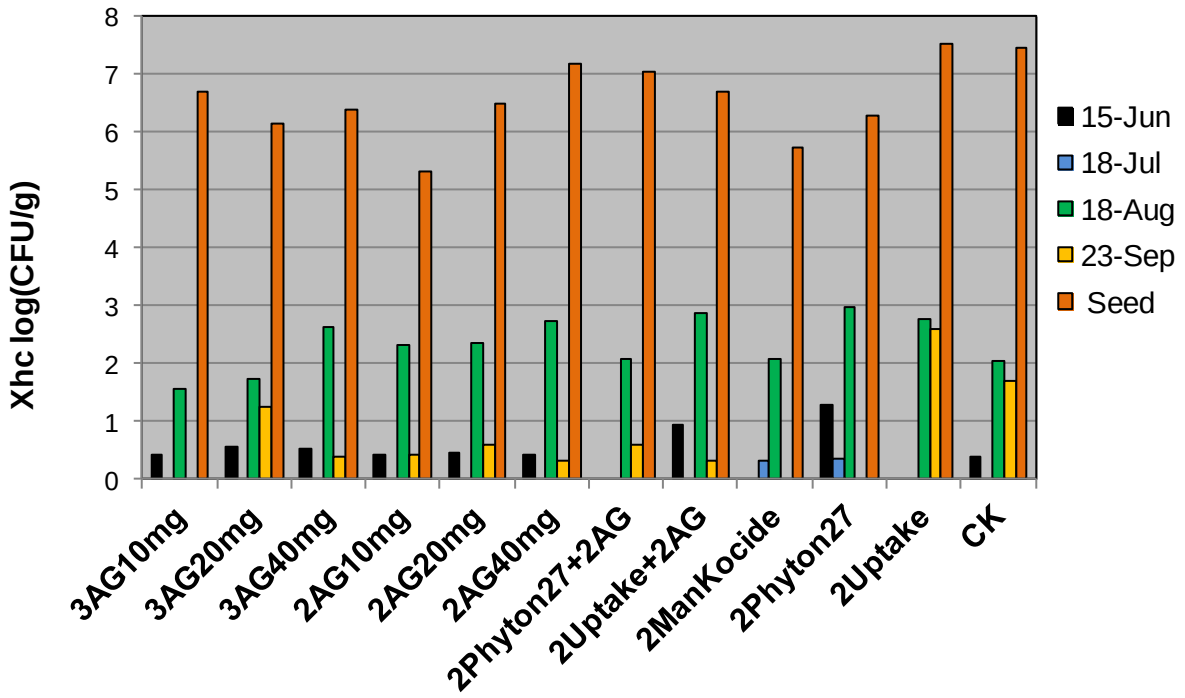

Fig. 1. Population level of *Xhc* on carrot plants and seeds subjected to different Actigard® (1, 2, and 3 times of Actigard-AG® at 10, 20 and 40 mg/plant) and chemical (Phyton 27®, Uptake® and ManKocide®) treatments in miniplot field trial 2011.

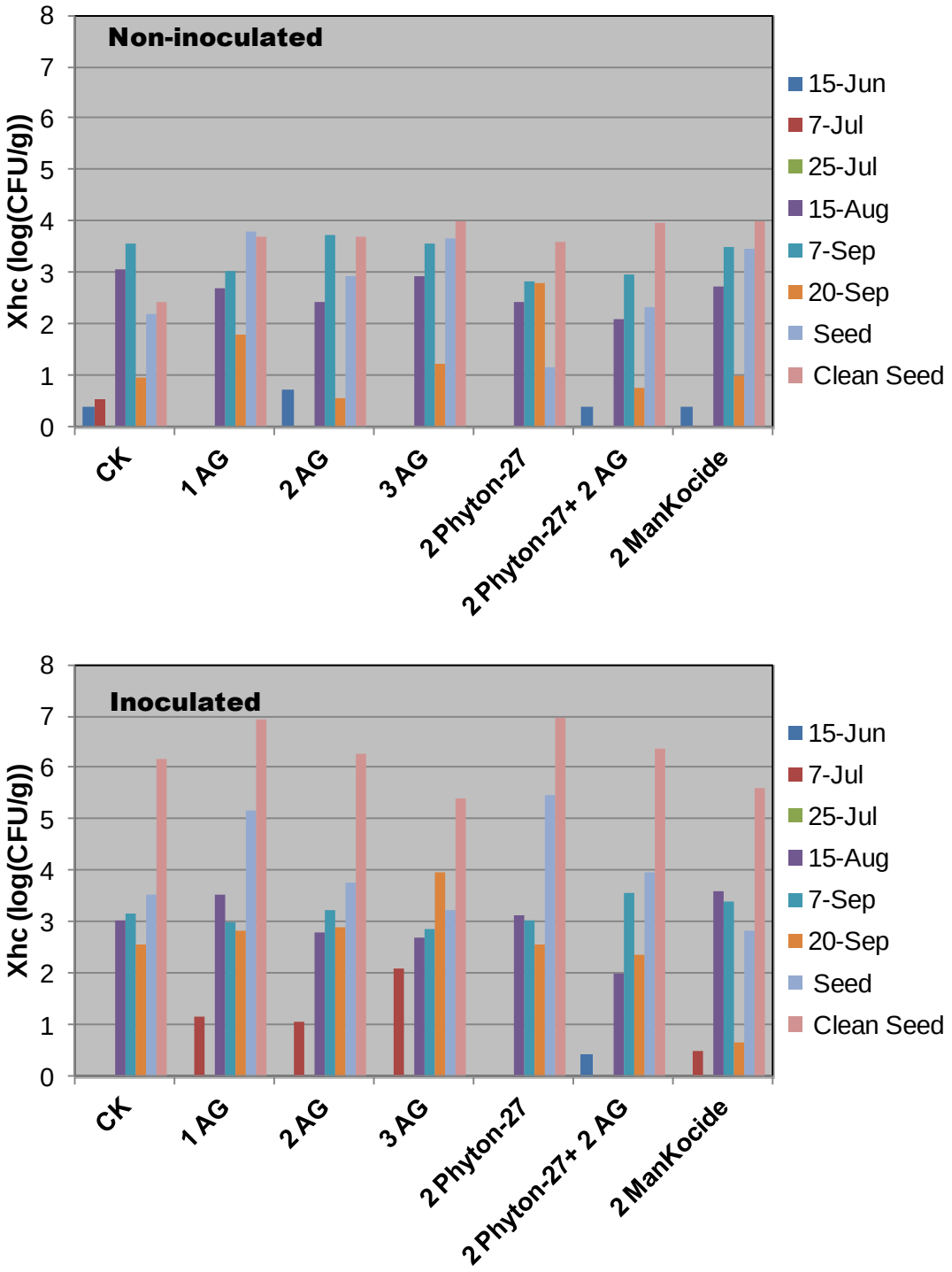

Fig. 2. Population level of *Xhc* on carrot plants and seeds subjected to different Actigard® (1, 2, and 3 times of Actigard-AG® applications) and chemical (Phyton 27® and ManKocide®) treatments in 2011 large-plot field trial.

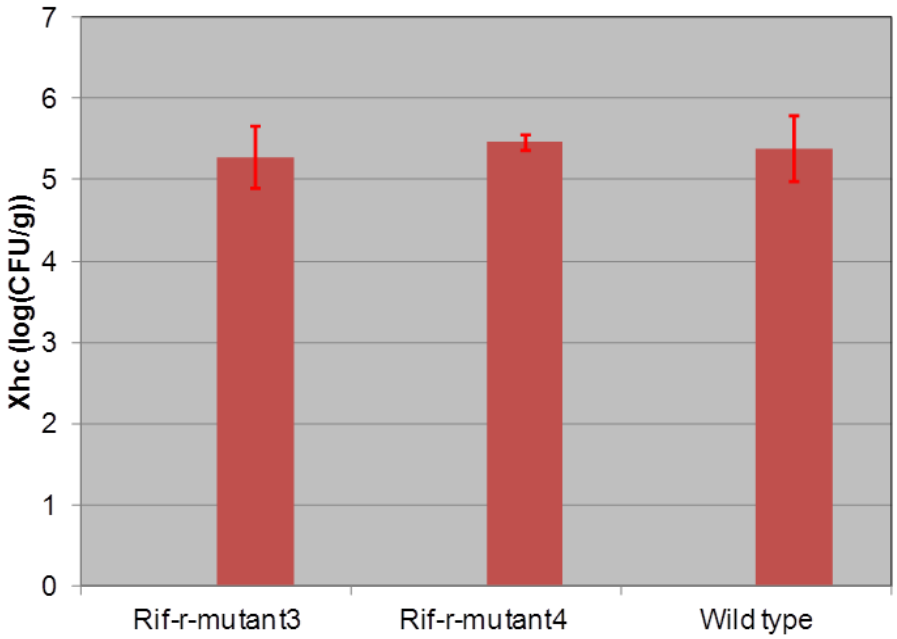

Fig. 3. Population level of Xhc on carrot seedlings treated with chemicals (Phyton 27®, Phyton 016B®, Uptake® and ManKocide®) in greenhouse experiments.

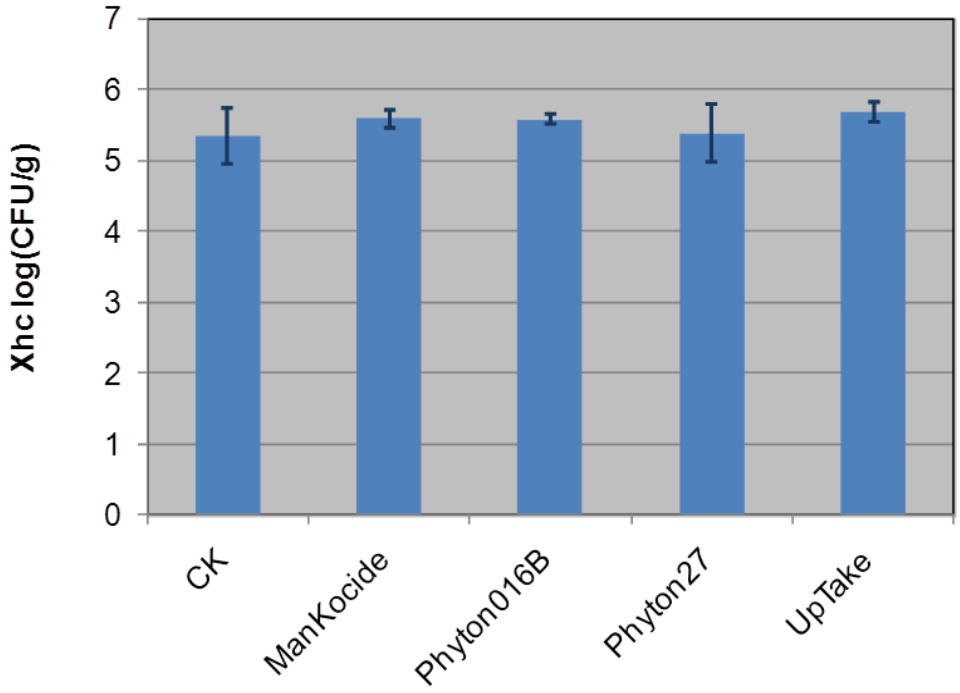

Fig. 4. Population levels of Xhc on carrot seedlings inoculated with wild type Xhc and further rifampicin resistant mutants 3 and 4.