Characterizing the Incidence and Distribution of Bacterial Blight Infestation in Individual Carrot Seeds: Can One Bad Seed Spoil the Whole Seed Lot?

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Introduction

Bulk samples of carrot seed are tested for *Xanthomonas hortorum* pv. *carotae* (*Xhc*) using a seed wash dilution-plating protocol (Asma, 2005). In this protocol, three 10 gram samples of carrot seed, equivalent to three subsamples of 10,000 seeds each, are soaked in buffer and serial dilutions are plated onto a semi-selective medium that limits the growth of bacteria other than *Xhc*. Testing protocols for seed-borne pathogens usually assume that infested seeds are fairly uniform (i.e. they follow the normal "bell-shaped curve") with regards to bacterial populations on individual seeds and that the assay will detect the average number of bacteria for infested seed present in the sample. However, several studies have shown that the number of bacteria found on individual seeds may vary widely and follow non-normal distributions (i.e. they do not follow the normal "bell-shaped curve") (Dutta et al., 2013). If the distributions of *Xhc* among infested carrot seeds are non-normal, assay results from bulk samples could result in an inaccurate estimate of the true population number. For example, if a seed lot contains relatively few, highly infested seeds, the bulk seed lot assay will be highly influenced by the number of highly infested seeds that are in a particular sample. On the other hand, a seed wash assay may not detect any infested seeds if only a few seeds in a seed lot are actually infested. The objective of this research is to determine the incidence and level of *Xhc* infestation among individual seeds in infested carrot seed lots. It is anticipated that this information will be important to the Oregon carrot seed industry, since many countries and markets have a zero-tolerance policy for *Xhc* in carrot seed. The incidence and level of infestation on individual seeds could also influence inoculum thresholds that are required for the development of bacterial blight in carrot root production. A better understanding of seed infestation in carrot seed lots will enable carrot seed producers to improve the methods used to prevent, detect, and treat infested seeds prior to market.

Materials and Methods

Carrot seed samples from commercial seed lots grown in central Oregon were subjected to a bulk seed wash dilution plate assay to determine the overall level of *Xhc*. Three 10 gram subsamples from each seed lot were soaked for 2 hours at room temperature in a 250 ml flask containing 100 ml of sterilized phosphate buffer and one drop of Tween 20 (a surfactant). After the soak the flasks were placed on a horizontal shaker set at 250 rpm for 5 minutes. A 10-fold dilution series (10^{-1} to 10^{-5}) was prepared for each subsample and each dilution series was plated onto replicated plates of semi-selective XCS agar medium. The plates were incubated at 82° F in the dark and monitored for the development of colonies typical of *Xhc*. The number of colonies typical of *Xhc* were counted after 5 to 7 days of incubation. Suspect colonies were sub-cultured onto diagnostic agar medium and subjected to a species-specific DNA test using polymerase chain reaction (PCR) to confirm their identities.

Individual seeds were assayed using a modification of the seed wash dilution plating assay described above. Single seeds were placed in wells of 96-well plates filled with phosphate buffer

and incubated for 2 hours at room temperature. Plates were shaken vigorously for 5 min on a horizontal shaker and 10-fold dilutions of the rinsate from each well was prepared, plated, and incubated as described above. The process was repeated until a minimum of 30 positive seeds were identified or 100 seeds were assayed.

Results and Discussion

A total of 16 seed lots were tested using bulk seed wash assay, all of which tested at or above the 10^5 CFU/g seed limit for *Xhc* (Fig. 1). However, results from the individual seed wash assays (828 seeds tested) indicate that the incidence of infested seed can vary among lots, ranging from 7.7 to 94.3% infested seed (Fig. 1). Seven of the 16 lots harbored infested seed at levels ≤ 20%, while four lots contained infested seed at an incidence of 23 to 39%. Among individual seed, the CFU detected ranged from 2 CFU (the limit of detection of the assay) to 6.4 x 10^6 CFU; three seed lots contained individual seeds with levels greater than 10^5 CFU (Fig. 2).

Among 11 seed lots and 548 seeds tested, *Xhc* was not detected on 68% of seed (Fig. 3). Among the remaining seed, 18% had low levels of *Xhc* (≤ 10 CFU), 8% of seed was infested at levels between 11 and 100 CFU, 4% of seed contained 101 to 1000 CFU, and 2% harbored >1000 CFU (Fig. 3). The distribution of infested seed in individual seed lots varied among seed lots but mostly reflected the higher distribution of non-infested seed (Fig. 4).

The results from this study to date indicate that seed infestation by *Xhc* is not homogenous in seed lots. In this study, *Xhc* was not detected from the majority (68%) of individual seeds assayed from commercial seed lots produced in central Oregon and 7 of the 16 seed lots tested contained 20% or less infested seed overall. However, the presence of a few, highly infested seeds in a seed lot may result in an unacceptable level of *Xhc* (≥10^5 CFU/g) in a bulk seed wash test. The epidemiological implications of a relatively few, highly infested seeds in a seed lot are not known, but the incidence of infested seed in a seed lot and infestation levels of individual seed may be important factors influencing seedborne transmission in carrot root crops. Highly infested seeds in seed lots may also be more difficult to disinfest using hot water or other seed treatments, especially when they represent relatively few seeds in a large seed lot.

Acknowledgements

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References

Asma, M. 2005. Proposal for a new method for detecting *Xanthomonas hortorum* pv. *carotae* on carrot seeds. ISTA Method Validation Reports 2: 1-17.

Dutta, B., C. Block, K. Stevenson, F. H. Sanders, R. Walcott and R. Gitaitis. 2013. Distribution of phytopathogenic bacteria in infested seeds. Seed Science and Technology 41: 383-397.

Figures

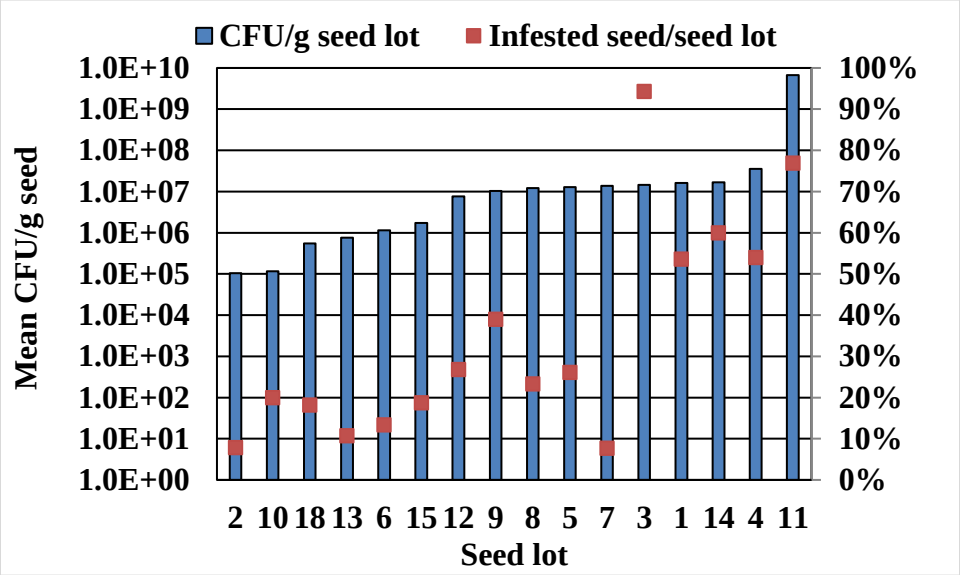

Fig. 1. Mean colony forming units (CFU) of the bacterial blight pathogen *Xanthomonas hortorum* pv. *carotae* in commercial carrot seed lots and percentage of individual carrot seed infested with the pathogen.

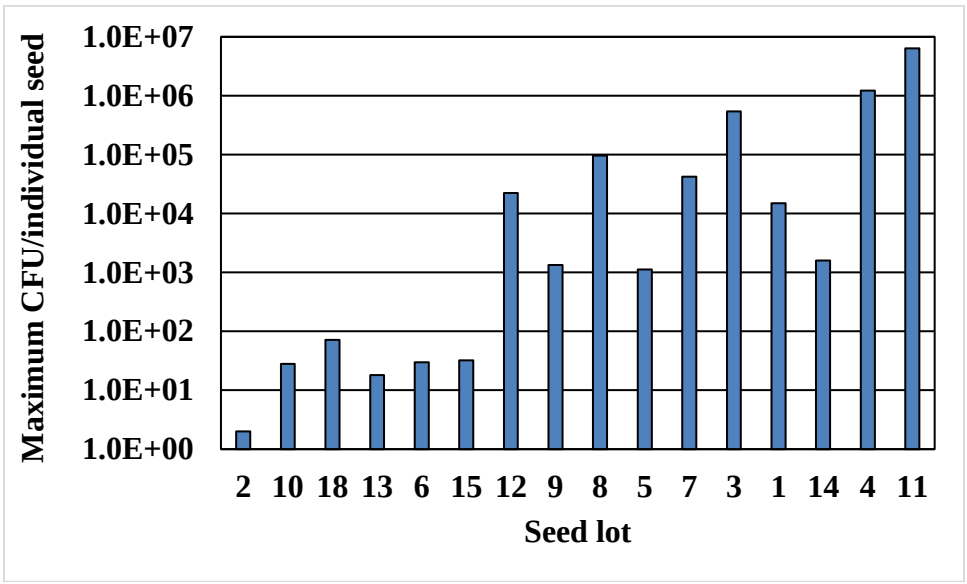

Fig. 2. The maximum colony forming units (CFU) of the bacterial blight pathogen *Xanthomonas hortorum* pv. *carotae* observed on an individual carrot seed from different commercial carrot seed lots.

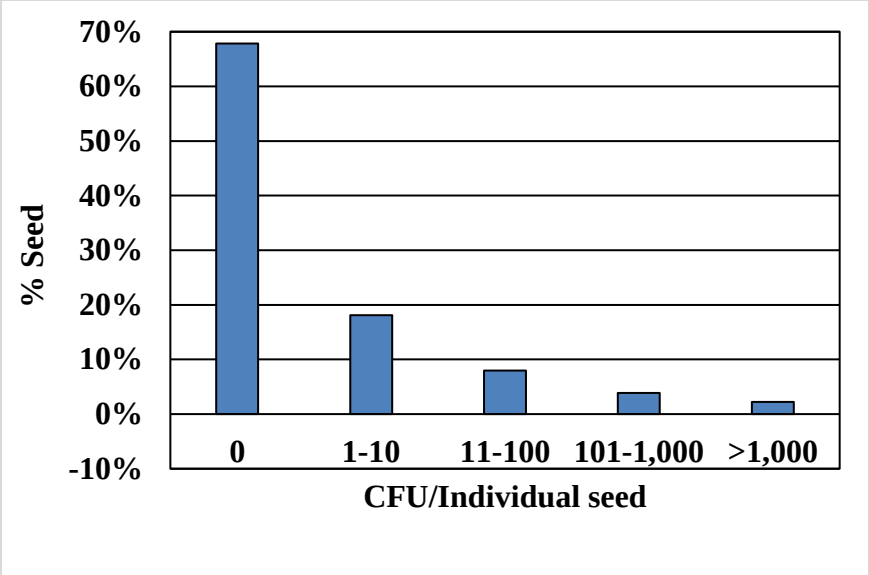

Fig. 3. Histogram showing the mean percentage of individual carrot seed harboring 0, 1 to 10, 11 to 100, 101 to 1,000, and over 1,000 colony forming units (CFU) of the bacterial blight pathogen *Xanthomonas hortorum* pv. *carotae*. A total of 548 seeds from 11 commercial seed lots were tested.

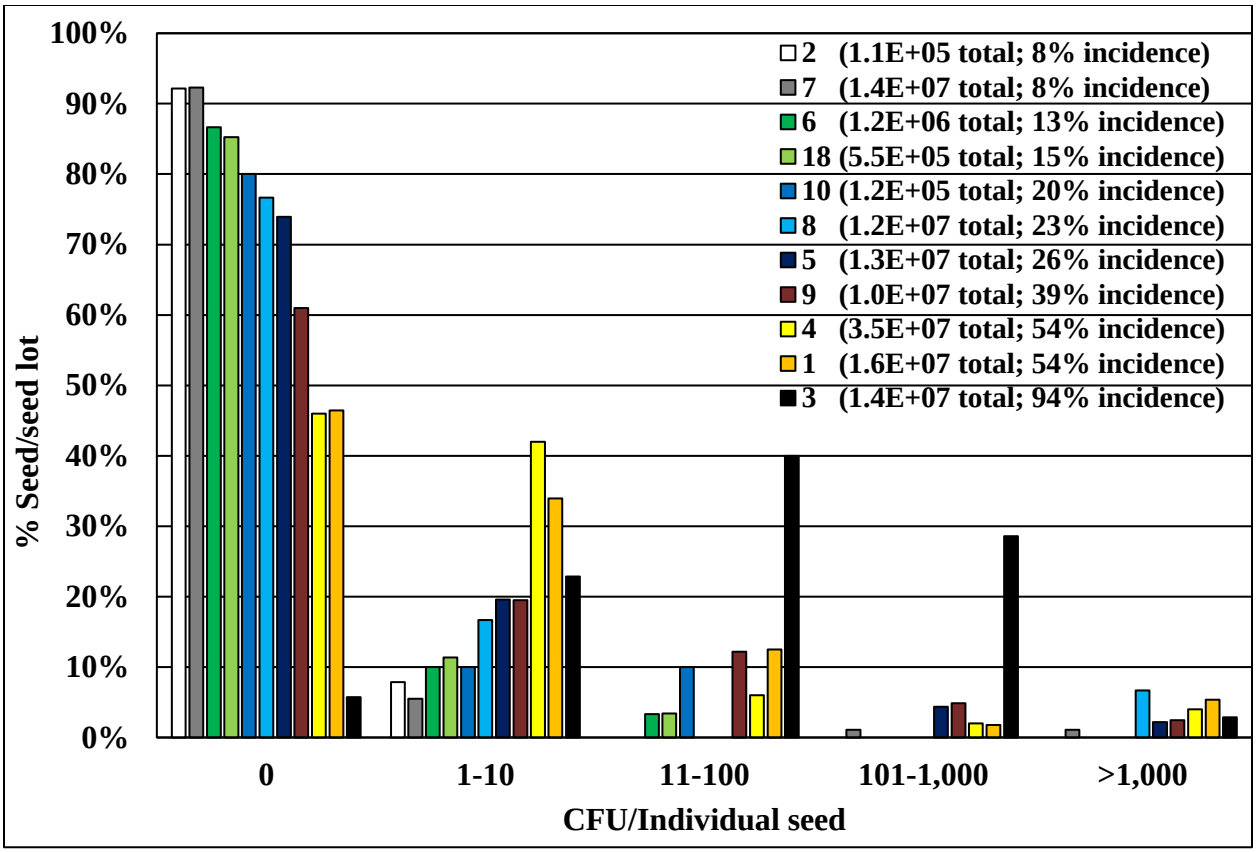

Fig. 4. Histogram showing the percentage of individual seed (*n* = 548) among 11 seed lots harboring 0, 1 to 10, 11 to 100, 101 to 1,000, and over 1,000 colony forming units (CFU) of the bacterial blight pathogen *Xanthomonas hortorum* pv. *carotae*. Legend indicates seed lot number, the total CFU level obtained from bulk seed wash assays, and the incidence of infected seed detected in individual seed assays.