Re-evaluating seed contamination and seed transmission thresholds for carrot bacterial blight

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Introduction

Existing seed contamination thresholds for *Xanthomonas* used by the carrot seed industry were developed using artificially-infested seed inoculated at a uniform rate and blended with healthy seed (Umesh et al. 1998). However, previous research revealed that the incidence and severity of *Xanthomonas*-contaminated seed can vary drastically within and among naturally-infested commercial carrot seed lots and, in most cases, a small proportion of seed is infested (Dung et al. 2016). Additionally, a relatively few number of seeds can harbor high populations of the pathogen ($>10^5$ CFU/seed). A re-evaluation of seed contamination and seed transmission thresholds is needed to better understand the epidemiological and market implications of these new findings. This project used naturally-infested seed to help determine: 1) the incidence of infested seed required for seed-borne transmission of bacterial blight; and 2) the level of *Xanthomonas* required on an individual seed to transmit the pathogen to carrot seedlings.

Materials and Methods

Objective 1. Five commercial carrot seed lots containing similar levels of overall *Xanthomonas* contamination (ranging from 1.3 to 4.9 x 10^8 CFU/g seed as determined in bulk seed wash assays) but different incidences of infested seed (27, 43, 56, 92, and 98% as determined in single-seed assays) were used in this study. The five seed lots represented two different proprietary hybrid carrot lines. Between 74 and 97 seeds from each lot were planted in flats containing peat-based greenhouse mix and placed in a growth chamber under conditions conducive for bacterial blight development (82/64° F day/night and 90-100% relative humidity). Flats were covered with clear plastic domes to maintain high relative humidity and reduce flat-to-flat contamination. Seedlings were harvested at the 2-3 leaf stage, bulked, and subjected to a leaf wash assay to determine the level of *Xanthomonas* on all seedlings in each flat. A total of 10 flat assays were performed among the five seed lots. *Xanthomonas* levels (CFU/100 seedlings) for each flat were log-transformed and subjected to correlation analysis.

Objective 2. Seeds from four commercial carrot seed lots representing three proprietary hybrid carrot lines were obtained. Carrot seeds were individually assayed to identify seeds with varying levels of natural infestation (ranging from 0 to 3.6 x 10^7 CFU/seed). The same seeds were then planted in 6-cell trays containing greenhouse potting mix and placed in a growth chamber (82/64° F day/night and 90-100% relative humidity). Flats were covered with clear plastic domes until germination. Seedlings were harvested at the 2-3 leaf stage and individually assayed to determine the level of *Xanthomonas* on each seedling. *Xanthomonas* counts (CFU/seedling) for each individual seeding were log-transformed and subjected to correlation analysis.

Results and Discussion

Objective 1. Five highly infested (10^8 CFU/g) seed lots with varying amounts of infested seed

(27-98% incidence) were planted in bulk under conditions highly conducive to bacterial blight. Although symptoms were not observed, *Xanthomonas* was recovered from 9 out of 10 plantings at relatively high levels (6.7×10^5 to 4.3×10^8). While not significant, stronger correlation with seedling infestation was observed for the incidence of infested seed ($r = 0.62$; $P = 0.07$) than the overall level of *Xanthomonas* in the seed lot as determined by bulk seed wash assays ($r = 0.33$; $P = 0.39$).

Objective 2. Previous studies have shown that, on average, 94% of carrot seeds from commercial seed lots contain < 100 CFUs per individual seed (Dung et al. 2016). Pathogen transmission from seed to seedling was not observed for individual seeds that harbored ≤ 10 CFU, and the pathogen was only detected on 9% of seedlings grown from individual seed with 11 to 100 CFU. Transmission of *Xanthomonas* from seed to seedling was greater in seed harboring larger pathogen populations, with pathogen transmission rates ranging between 20% (for seed containing 10^2 to 10^3 CFU/seed) to 67% (for seed containing 10^4 to 10^5 CFU/seed). These results suggest that seed with < 100 CFUs of *Xanthomonas* may not be as important for seedborne transmission as seed with greater levels (>10^2 CFU) of *Xanthomonas*.

Overall germination was lower, which may have been due to the seed wash assay that each seed was subjected to prior to planting. Regardless, germination appeared to be negatively impacted by greater levels of seed infestation. Germination rates for seed harboring between 10^2 and 10^6 CFU ranged from 50 to 71% compared to seed with lower levels of the pathogen (Table 1).

Highly infested individual seeds were less frequent in commercial seed lots, so additional data points are needed to better characterize seedborne transmission from seeds with higher levels (>10^3 CFUs) of the pathogen. The effect of *Xanthomonas* contamination on seed germination may also warrant further investigation. It should be noted that these initial studies were performed under conditions highly conducive for bacterial blight development and additional studies should be conducted to determine the effect of environment on seed-borne transmission of bacterial blight.

Acknowledgements

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References

Dung, J., Scott, J., and Weber, M. 2016. Characterizing the incidence and distribution of bacterial blight infestations in individual carrot seeds: Can one bad seed spoil the whole lot? Central Oregon Agricultural Research Center 2016 Annual Report: 22-26.

Umesh, K. C., Davis, R. M., and Gilbertson, R. L. 1998. Seed contamination thresholds for development of carrot bacterial blight caused by *Xanthomonas campestris* pv. *carotae*. Plant Disease 82(11):1271-1275.

Tables and Figures

Fig. 1. Correlation between *Xanthomonas hortorum* pv. *carotae* (*Xhc*) levels in 10 g seed lot samples and pathogen populations on seedlings grown from the same seed lots.

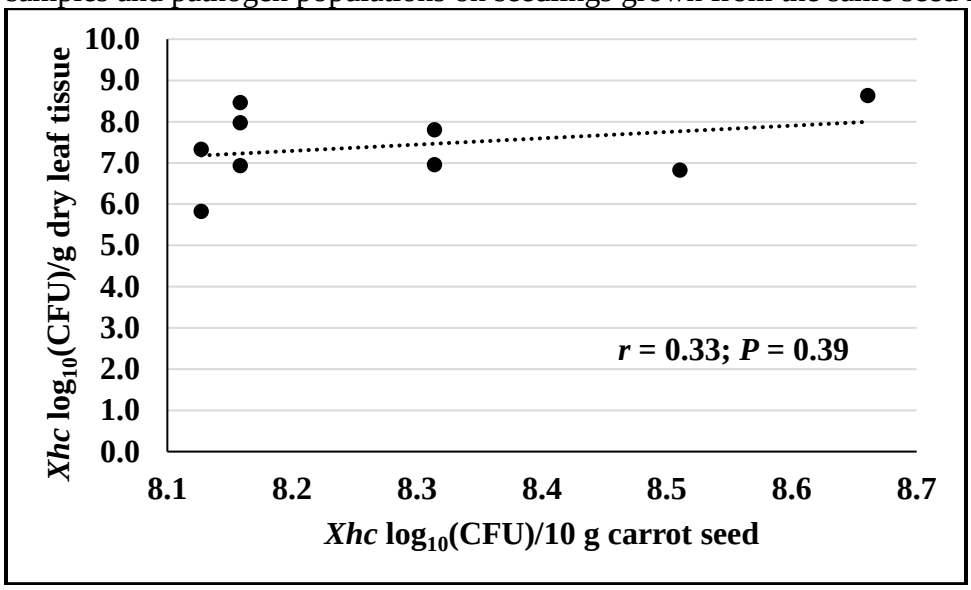

Fig. 2. Correlation between the incidence of seed infested with *Xanthomonas hortorum* pv. *carotae* (*Xhc*) and pathogen populations on seedlings grown from the same seed lots.

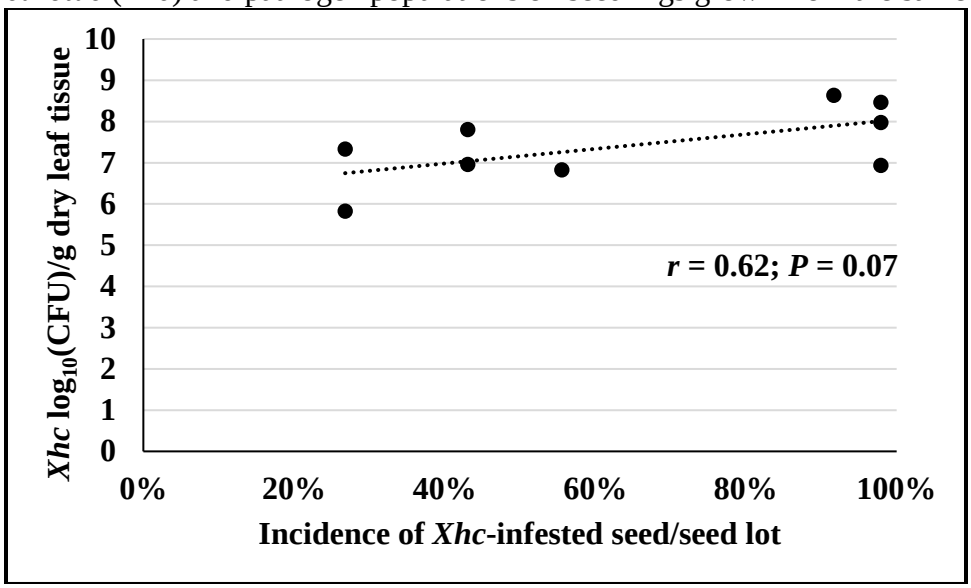

Table 1. Percent germination, percent pathogen transmission, and mean colony forming units (CFU) on seedlings grown from individual carrot seeds with varying levels of *Xanthomonas hortorum* pv. *carotae* infestation.

***Xanthomonas* CFU/individual seed**	***n***	**Germination**	**Pathogen transmission to seedlings**	**Mean CFU/ seedling**
0	**51**	80%	0%	0
1-10	**45**	78%	0%	0
11-100	**68**	82%	9%	4.7×10^4
101-1,000	**23**	74%	20%	9.5×10^5
1,001-10,000	**8**	63%	40%	9.4×10^6
10,001-100,000	**4**	50%	67%	1.9×10^7
100,001-1,000,000	**7**	71%	60%	1.5×10^7
> 1,000,000	**8**	50%	25%	1.2×10^4