# Screening for Resistance to *Xanthomonas hortorum* pv. *carotae* in *Daucus carota*

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## Abstract (36th International Carrot Conference, 14-17 August 2013, Madison, WI)

*Xanthomonas hortorum* pv. *carotae* causes bacterial blight of carrot. Genetic resistance to bacterial blight is limited in commercial carrot cultivars, and little public research has been carried out on screening for sources of resistance. Furthermore, disease symptoms typically do not develop until pathogen populations reach $10^5$ to $10^6$ CFU/g dry leaf tissue, complicating evaluations for resistance to *X. hortorum* pv. *carotae* infections in the field. Sixty-six plant introduction (PI) lines from the United States Department of Agriculture (USDA) National Plant Germplasm System, two inbred carrot lines from Dr. Philipp Simon's carrot breeding program at the University of Wisconsin, and 17 proprietary carrot hybrids or open pollinated lines from six vegetable seed companies or breeders were screened for resistance to *X. hortorum* pv. *carotae*. Evaluations included percentage of the foliage that developed symptoms six weeks post-inoculation, and population of the pathogen (CFU/g dry leaf tissue) detected on a semi-selective agar medium (XCS) when the foliage was assayed six weeks post-inoculation. Pathogen populations on foliage ranged from 1.38 X $10^4$ to 3.28 X $10^{11}$ CFU/g dry leaf tissue for individual plants, with a median of 2.80 X $10^9$ CFU/g dry leaf tissue, and a mean ± standard error of 8.16 X $10^9$ ± 1.07 X $10^9$ CFU/g dry leaf tissue. Severity of symptoms ranged from 0 to 50% of the foliage blighted, with a median of 7% and a mean of 8.84 ± 0.38%. Visual symptoms after 6 weeks and CFU/g dry leaf tissue (arcsine transformed) were significantly correlated (Pearson's correlation coefficient of r = 0.4685 at P<0.0001). Eight PI lines with potential resistance (lowest visual ratings and populations of *X. hortorum* pv. carotae detected) were selected for a second screening, along with five of the most susceptible PI lines to aid in the study of inheritance of resistance or susceptibility to *X. hortorum* pv. *carotae*. The lines that express the most resistance in the second screening will be crossed to inbred carrot lines from Dr. Simon's program in an effort to incorporate the resistance into a genetic background available for public use. The variation detected among entries in this study suggests it should be possible to develop carrot cultivars with improved resistance to bacterial blight, but resistance is likely to be highly quantitative. Screening a more diverse set of *Daucus* germplasm may be necessary to identify greater sources of resistance.

## Background

Worldwide, *Daucus carota* (carrot) root crops are grown on up to 1.2 million ha annually with up to 28 million metric tons harvested (5). In the Pacific Northwest (PNW) U.S. (WA, ID, and OR), carrot seed and root crops are important commodities. PNW carrot seed crops account for ≤50% of the world supply and 75% of the U.S. supply of carrot seed (4). Furthermore, Washington carrot growers produced the first- or second-most tonnage of processing carrots in the U.S. since processing carrot production records have been mainained (5). Despite different practices for carrot seed and root crops, they are subject to the same diseases. Root crops are harvested primarily by pulling the tops, so leaf blights, e.g., bacterial blight caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*), can cause significant losses at harvest **(Fig. 1)**. Bacterial blight is a problem wherever carrots are grown (3). *Xhc* is spread by seed, on crop debris during harvest, by splashing water, and possibly by insects (2,3). du Toit et al. (2) showed that *Xhc* is prevalent in carrot seed crops in the PNW, readily infecting harvested seed. Carrot seed crops are grown in the southern and northern hemispheres, and seed- companies must meet thresholds of seedborne *Xhc* for international seed trade. For example, the Mexican government recemtly implemented a zero tolerance for *Xhc* on carrot seed imports. Although variation in susceptibility to bacterial blight has been documented among cultivars, resistance to bacterial blight is limited in commercial carrot cultivars and there has been little public research on screening for sources of resistance to *Xhc* (1).

## Results

- 454 plants were assayed for reaction to *Xhc* (**Fig. 2**)
- Severity of bacterial blight symptoms 6 weeks after inoculation ranged from 0 to 50% foliar blighting (median of 7.00% and mean ± standard error of 8.84 ± 0.38%) (**Table 1**).
- *Xhc* populations ranged from 1.38 X $10^4$ to 3.28 X $10^{11}$ CFU/g dry leaf tissue for individual plants, with a median of 2.80 X $10^9$ CFU/g and a mean of 8.16 X $10^9$ ± 1.07 X $10^9$ CFU/g (**Table 1**).
- The population of *Xhc* and foliar ratings differed significantly among lines, but also among individual plants within lines, reflecting variability typical of this pathogen (2,3).
- The population of *Xhc* recovered was positively correlated with foliar severity ratings (r = 0.4685 at P<0.0001) (**Fig. 3**).
- Eight PI lines with potential resistance to *Xhc* (lowest foliar severity ratings, from 0 to 20%; and smallest population of *Xhc* recovered, from 1.38 X $10^4$ to 4.36 X $10^9$) were selected for a second screening, along with the five most susceptible PI lines (severity ratings of 0 to 50%, and *Xhc* population from 7.06 X $10^8$ to 2.34 X $10^{11}$) to aid in evaluating potential inheritance of resistance or susceptibility (**Table 1**).
- 8 putative resistant PIs: 163238, 176969, 177381, 263601,418967, 432905, 432906, and 436674.
- 5 susceptible PIs: 226636, 234621, 277710, 390893, and 390887.

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Fig. 1: Carrot bacterial blight symptoms.

Fig. 2: Inoculating carrot foliage with *X. hortorum* pv. *carotae*.

Table 1: Evaluation of carrot PI lines, inbreds, and commercial cultivars.

| Line | Foliar Rating (%) | CFUs/g dry leaf tissue |
|---|---|---|
| PI 418967 | 7.4 | $9.27x10^6$ |
| PI 432905 | 3.6 | $2.50x10^7$ |
| PI 176969 | 2 | $1.39x10^8$ |
| PI 263601 | 3.2 | $6.73x10^8$ |
| PI 432906 | 4.6 | $4.85x10^8$ |
| PI 436674 | 8 | $1.10x10^9$ |
| PI 177381 | 3 | $1.06x10^9$ |
| PI 163238 | 8 | $1.15x10^9$ |
| PI 226636 | 12.4 | $3.65x10^{10}$ |
| PI 390887 | 22.8 | $6.17x10^{10}$ |
| PI 390893 | 20.4 | $2.69x10^{10}$ |
| PI 234621 | 16 | $1.55x10^{10}$ |
| PI 277710 | 10.4 | $1.75x10^{10}$ |
| WISC2566A | 5.95 | $8.68x10^9$ |
| WISC0493A | 4.75 | $1.66x10^9$ |
| Open Pollinated1 | 19 | $1.21x10^{10}$ |
| Open Pollinated2 | 11 | $1.36x10^{11}$ |
| Open Pollinated3 | 11.4 | $4.68x10^9$ |
| Hybrid1 | 11 | $6.91x10^{10}$ |
| Hybrid2 | 8.6 | $9.16x10^9$ |
| Hybrid3 | 4.4 | $2.27x10^9$ |
| Hybrid4 | 5 | $1.09x10^9$ |
| Hybrid5 | 6 | $1.03x10^9$ |
| Hybrid6 | 9.8 | $4.49x10^9$ |
| Hybrid7 | 4.6 | $4.74x10^8$ |
| Hybrid8 | 7 | $5.31x10^9$ |
| Hybrid9 | 9 | $4.68x10^9$ |
| Hybrid10 | 10.4 | $1.75x10^{10}$ |
| Hybrid11 | 8 | $1.50x10^{10}$ |
| Hybrid12 | 13 | $8.15x10^9$ |
| Hybrid13 | 7 | $1.05x10^{10}$ |
| Hybrid14 | 4.6 | $2.29x10^9$ |

Red=susceptiple; Green=resistant

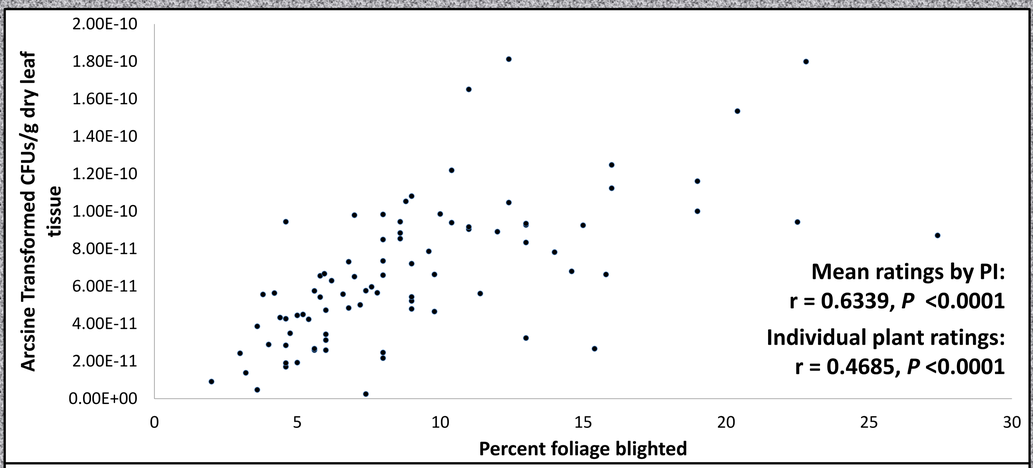


Fig. 3: Correlation between mean foliar ratings and mean amount of *Xanthomonas hortorum* pv. *carotae* detected on carrot PI lines.

## Materials and Methods

Seed of carrot Plant Introduction (PI) lines were obtained from the USDA North Central Plant Introduction Station (Ames, IA) as well as two inbred, male sterile carrot lines (Phil Simon, USDA ARS, University of Wisconsin), and 17 proprietary cultivars. PI lines were selected based on preliminary screening for reaction to *Xhc* by cooperating seed companies.

Plants were grown in a greenhouse at the Washington State University Northwestern Washington Research and Extension Center, in a randomized layout of 25 plants/rack. Ten weeks after planting, plants were enclosed in clear plastic bags for 24 h to increase relative humidity, the foliage spray-inoculated with *Xhc* (~$10^8$ CFU/ml) until runoff (**Fig. 2**), and enclosed in bags for 72 h.

Foliar bacterial blight severity ratings (% of foliage with symptoms) were done for all plants/line 4 and 5weeks post-inoculation. Five individual plants were then rated/line 6 weeks post-inoculation. After this rating, the population of *Xhc* on the foliage was quantified by assaying five individual plants/line (2). The foliage of each plant was cut into small pieces, shaken in $PO_4$ buffer for 1 h, and a 10-fold dilution series prepared. An aliquot of each dilution was plated onto the semi-selective XCS agar medium (3 replicate aliquots/dilution), the plates incubated at 28°C for 5 days in the dark, and colony counts calculated/g dry weight of the assayed foliage.

## Discussion

- This screening demonstrated a wide range in reaction of 66 PIs to *Xhc* under greenhouse conditions.
- Variation among PIs in foliar ratings and *Xhc* levels detected suggests it may be possible to select and incorporate greater resistance to bacterial blight into commercial cultivars.
- However, variation in foliar ratings and *Xhc* populations among plants of individual lines demonstrated how erratic development of bacterial blight can complicate efforts to screen for resistance (3), even with plants inoculated uniformly.
- This, combined with the heterogeneous PIs, means multiple screenings are needed to improve confidence in the results.
- Following a second screening, crosses will be made between putative resistant PIs and two inbred, male sterile lines, as well as susceptible PIs and the inbreds, to determine the inheritance of resistance/susceptibility.
- Progeny populations will be developed and screened in field trials to assess if greenhouse results reflect reactions in field conditions.

### References

1. Boiteux, L.S., & P.W. Simon. 2002. Breeding for disease resistance in carrots. Pp. 7-8 in: Compendium of Umbelliferous Crop Diseases. R. M. Davis & R. N. Raid, Editors. American Phytopathological Society, St. Paul, MN.
2. du Toit, L.J., Crowe, F.J., Derie, M.L., Simmons, R.B., & Pelter, G.Q. 2005. Bacterial blight in carrot seed crops in the Pacific Northwest. Plant Dis. 89:896-907.
3. Gilbertson, R. L. 2002. Bacterial leaf blight of carrot. Pp. 11-12 in: Compendium of Umbelliferous Crop Diseases. R. M. Davis & R. N. Raid, Editors. American Phytopathological Society, St. Paul, MN.
4. Thomas, J., Schreiber, A., Pelter, G. Q., & Havens, D. 1997. Washington's Small-Seeded Vegetable Seed Industry. Washington State University Ext. Bull. No. 1829.
5. United States Department of Agriculture. 2011. U.S. Carrot Statistics. Washington, DC, USA: Agricultural Research Service, USDA. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1577 [2012].
6. United States Department of Agriculture. 2012. U.S. Carrot Statistics. Washington, DC, USA: National Agricultural Statistics Service, USDA. http://usda01.library.cornell.edu/usda/current/VegeSumm/VegeSumm-01-29-2013.pdf