The effect of seedborne *Xanthomonas hortorum* pv. *carotae* on seed germination and seedborne transmission of bacterial blight in carrot

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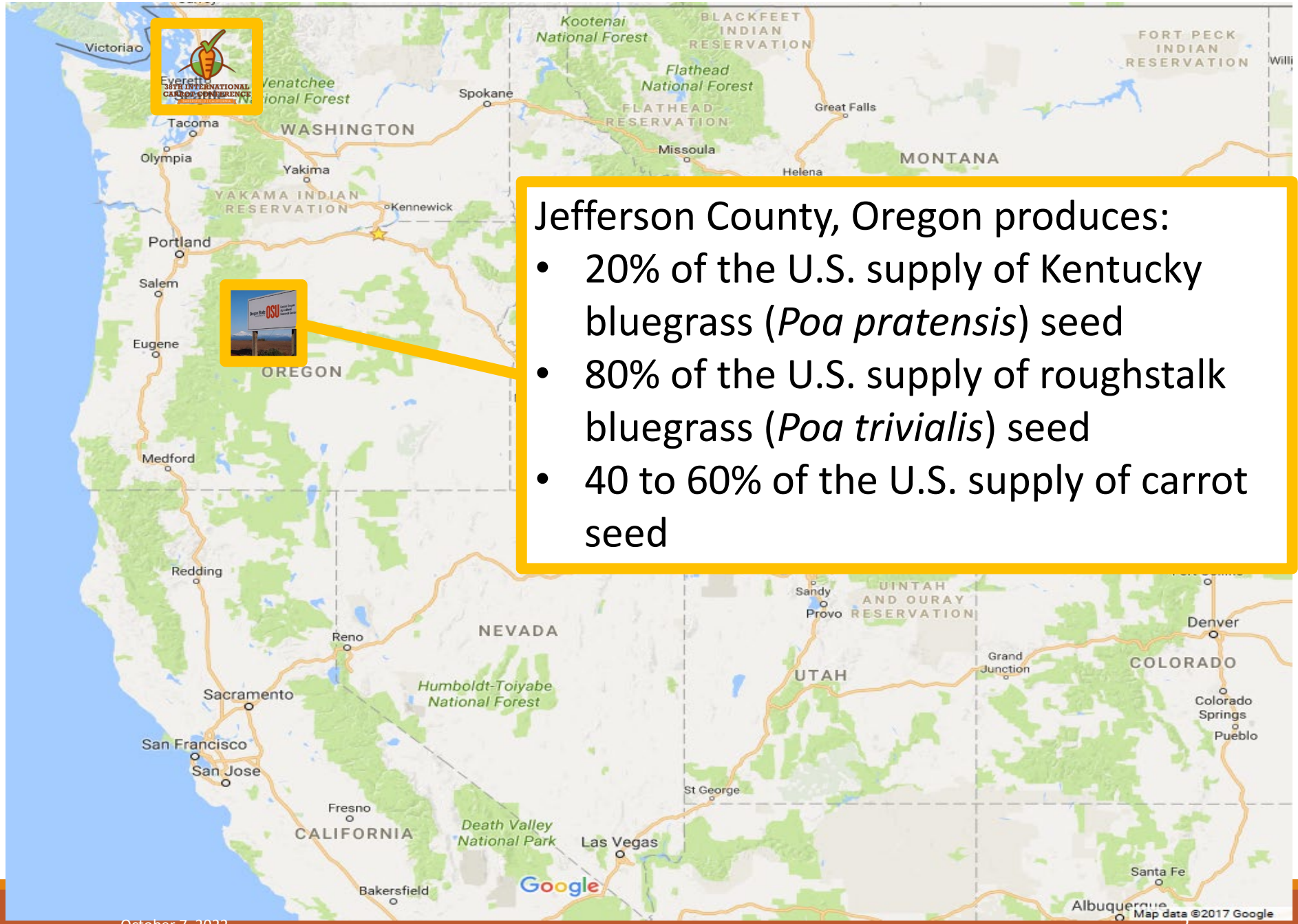


Jefferson County, Oregon produces:

- 20% of the U.S. supply of Kentucky bluegrass (*Poa pratensis*) seed
- 80% of the U.S. supply of roughstalk bluegrass (*Poa trivialis*) seed
- 40 to 60% of the U.S. supply of carrot seed

Agricultural Crop Production in Central Oregon (2020)

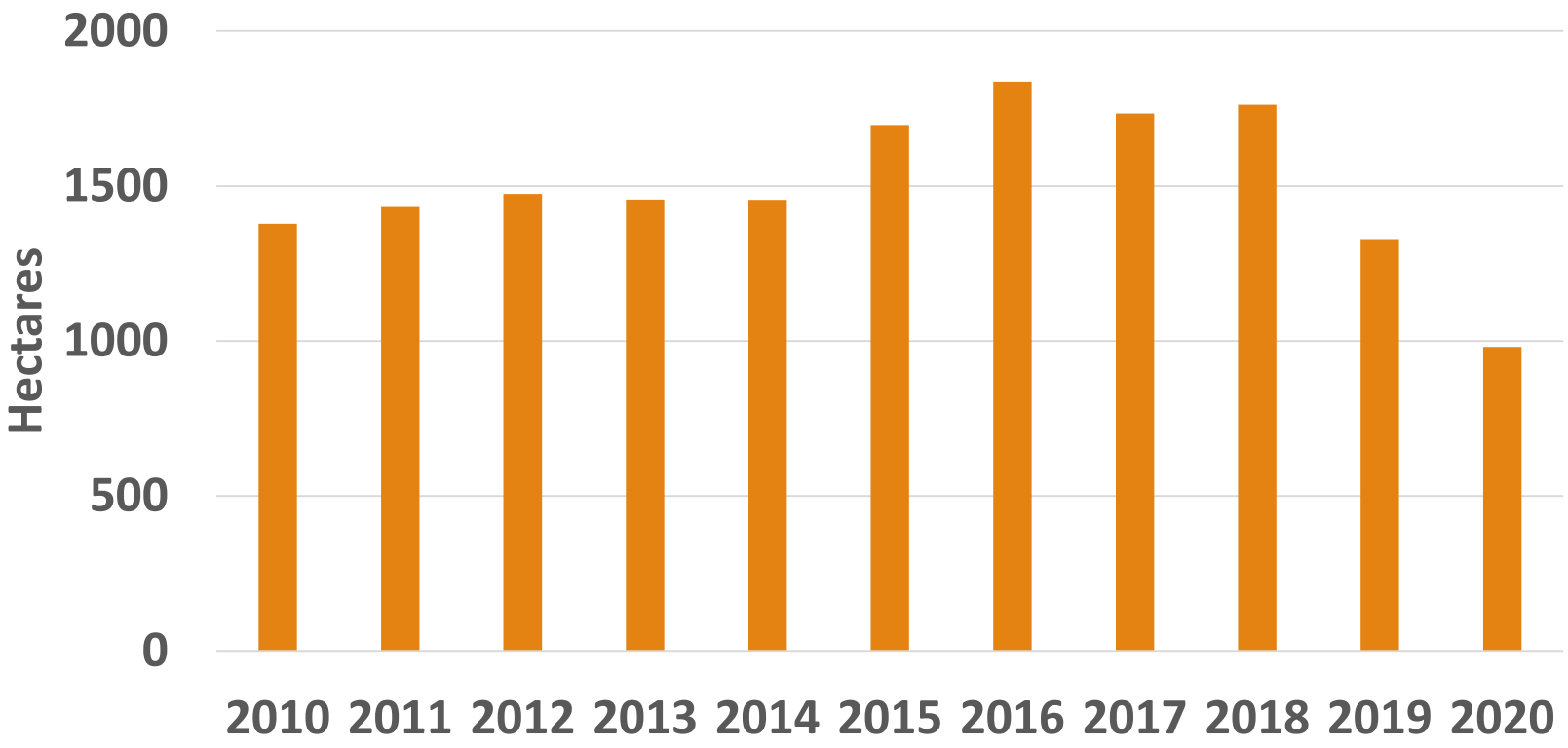
Seed crops	Hectares
Grass	5,097
Carrot	981
Garlic	49
Radish	159
Potato	170
Flowers	88
Mustard	102
Wheat	65
Triticale	55

Cereals and forage	Hectares
Alfalfa hay	3,983
Other hay	3,137
Irrigated pasture	3,422
Grain hay	580

Selected misc.	Hectares
Peppermint	227
Spearmint	61
Cilantro	62
Hemp	174
Pumpkins	74

Source: North Unit Irrigation District Crop Report

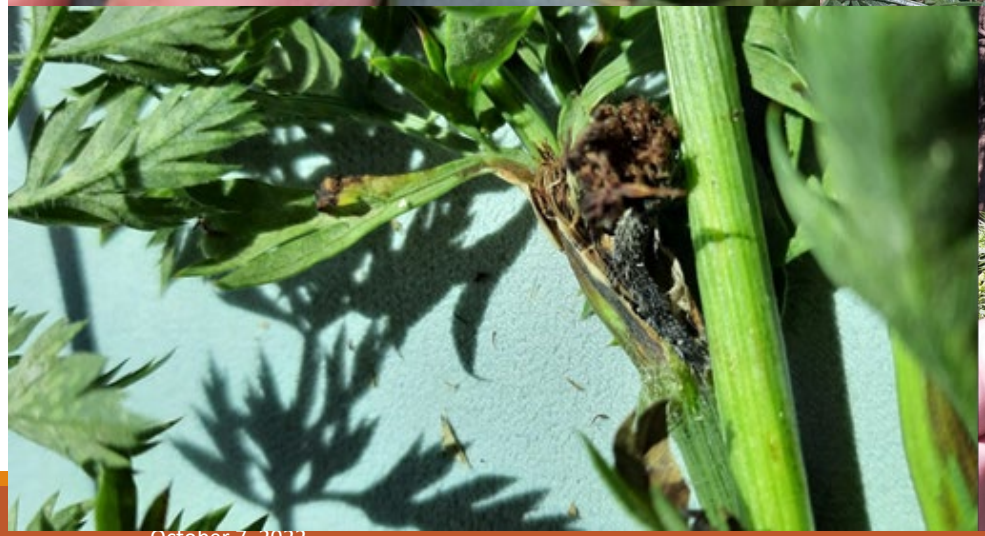
Carrot Seed Production in Central Oregon



Source: North Unit Irrigation District Crop Report

Bacterial Blight of Carrot

- Caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*)
- CA (1931), AZ, NM, MI, FL, NY, WI, ID, WA, OR
- Infects leaves, petioles, umbels, seed
- Survives and reproduces epiphytically
- Large populations ($\geq 10^6$ CFU/g leaf tissue) required for symptom development
- Yellow, angular spots that expand into irregular, brown, water-soaked lesions surrounded by a yellow halo



Bacterial Blight in Carrot Seed Production

- Seed- and steckling-borne
- Dry climate and drip irrigation limit direct impact of *Xhc* on seed crops
- Umbel blights can reduce seed yield
- Infested seed usually necessitates hot water treatment

Seedborne Transmission of Bacterial Blight in Carrot

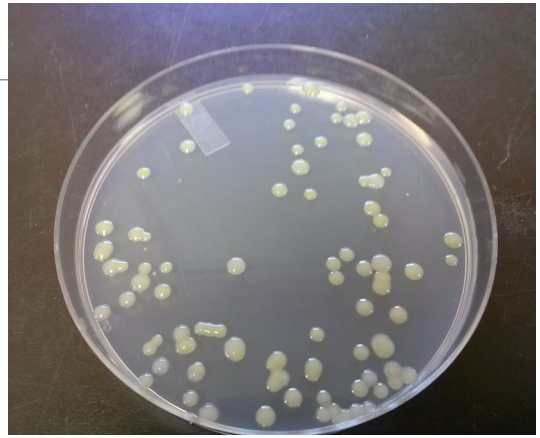
- Seed-transmitted at 10^4 CFU/g seed (Umesh et al. 1998)
  - Internal or external contamination
  - 10^5 to 10^7 CFU/g seed required for epidemic development
  - Under arid, overhead irrigated conditions in CA

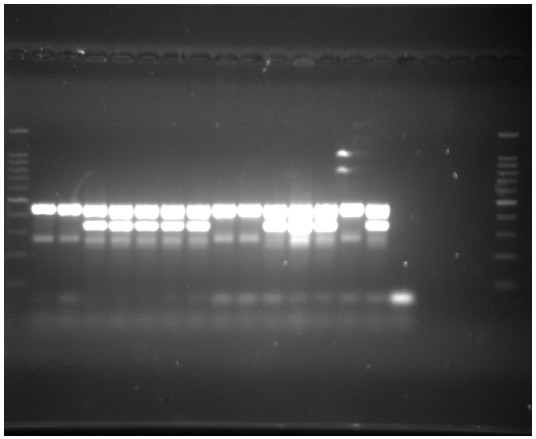
- Study used artificially-infested seed inoculated at a uniform rate and blended with healthy seed

- Recent research revealed that the incidence and severity of *Xhc*-contaminated seed can vary within and among naturally-infested commercial carrot seed lots
  - Plant Disease 104(8): 2144-2148. doi.org/10.1094/PDIS-12-19-2674-RE.

Materials and Methods

- 24 seed lots produced in central OR between 2014 and 2016
- Overall *Xhc* levels determined using a bulk seed wash assay (du Toit et al. 2017)
  - (3) 10 g subsamples of seed soaked in PO_4 buffer + Tween
  - Plated ten-fold dilutions (10^0 to 10^{-5}) onto XCS agar and incubated at 28°C for 7 days
  - Suspect colonies confirmed via 3S PCR (Meng et al. 2004)
  - Limit of detection (LOD) = 100 CFU/g seed





Materials and Methods

- Individual seeds were assayed using a modification of the bulk seed wash assay
- Seeds individually placed in 96-deep well plates filled with PO_4 buffer + Tween
- Plating, incubation, and colony identification as previously described
- Assayed up to 30 (+) seeds or 100 seeds total
- LOD = 2 CFU/seed

Xhc CFU in Seed Lot and Individual Seed Samples

		Bulk (10-g) seed assay[b]		Individual seed assay[c]		
Year	*N*[a]	Mean	SD	Pos (%)	Mean	Max
Nontransformed						
2014	10	1.17E+07	4.02E+06	34.0%	4.70E+03	1.92E+05
2015	7	9.56E+08	1.56E+09	26.4%	3.79E+04	9.22E+05
2016	7	2.47E+08	1.19E+06	60.2%	1.92E+05	7.33E+06
Total	24	3.56E+08	4.57E+08	39.4%	6.89E+04	2.49E+06

Plant Disease 104(8): 2144-2148. doi.org/10.1094/PDIS-12-19-2674-RE.

Xhc CFU in Individual Seed Samples Varies Within a Seed Lot

Year, seed lot	N[b]	Minimum	Maximum	Mean	Median	SD[c]
Nontransformed						
2014						
1	30	2.0E+00	1.5E+04	6.0E+02	6.0E+00	2.7E+03
2	4	2.0E+00	2.0E+00	2.0E+00	2.0E+00	0.0E+00
3	33	2.0E+00	5.4E+05	1.6E+04	7.0E+01	9.4E+04
4	28	2.0E+00	1.2E+06	4.6E+04	6.0E+00	2.3E+05
5	12	2.0E+00	1.1E+03	1.6E+02	4.0E+00	3.4E+02
6	4	2.0E+00	3.0E+01	1.0E+01	4.0E+00	1.3E+01
7	7	2.0E+00	4.2E+04	6.1E+03	4.0E+00	1.6E+04
8	7	2.0E+00	9.6E+04	2.2E+04	2.0E+00	3.9E+04
9	16	2.0E+00	1.3E+03	1.3E+02	1.1E+01	3.4E+02
10	6	2.0E+00	2.8E+01	1.1E+01	6.0E+00	1.1E+01

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Seedborne Transmission of Bacterial Blight in Carrot

- In many cases, a small proportion of seed is infested
- In some cases a relatively few number of seeds can harbor high populations of the pathogen
- A re-evaluation of seed contamination and seed transmission thresholds may improve our understanding of seedborne transmission of *Xhc*
- The objective of this research was to determine the level of *Xanthomonas* required on an individual seed to transmit the pathogen to carrot seedlings

Materials and Methods

- 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 3.6×10^7 CFU/seed)
- The same seeds were then planted in 6-cell trays containing greenhouse potting mix and placed in a growth chamber (27.8/17.8°C day/night and 90-100% relative humidity)
- Seedlings were harvested at the 2-3 leaf stage and individually assayed to determine the level of *Xanthomonas* on each seedling

Effect of Seedborne *Xhc* on Seed Germination and Pathogen Transmission to Seedlings

Xhc 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

Conclusions

- Pathogen transmission from seed to seedling was not observed for individual seeds that harbored ≤ 10 CFU
  - The pathogen was detected on 9% of seedlings grown from individual seed with 11 to 100 CFU
  - *Xhc* transmission was greater for seed containing 10^2 to 10^3 CFU/seed (20%) and for seed containing 10^4 to 10^5 CFU/seed (up to 67%)
- Results suggest that seed with < 10 CFUs of *Xhc* may not be as important for seedborne transmission as seed with greater levels ($\geq 10^2$ CFU) of *Xhc*
  - Additional data are needed to better characterize seedborne transmission from seeds with higher levels ($>10^3$ CFUs) of *Xhc*
- Germination was negatively impacted by greater levels of seed infestation
- Information can be used to enhance seed lot sampling, testing, and treatment

Acknowledgements

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Thank you…Questions?