BACTERIAL BLIGHT OF CARROT SEED CROPS IN THE PACIFIC NORTHWEST: IDENTIFICATION OF SOURCES OF INOCULUM

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Introduction

Carrot seed crops in central Washington (WA) and central Oregon (OR) produce approximately 75 percent of the U.S. carrot seed (Thomas et al., 1997) on 2,000-3,000 acres per state (Pelter 2001). Bacterial blight, caused by *Xanthomonas campestris* pv. *carotae*, is a seed-borne disease of concern to the carrot seed industry in the Pacific Northwest and to carrot growers in many regions of the United States and the world. Infection of carrot seed by *X. campestris* pv. *carotae* may reduce seed germination, resulting in losses to seed growers if germination is <85 percent. Seed companies, in turn, face expenses associated with treating infected seed lots, and seed lots destined for export markets may be rejected. Bacterial blight continues to cause losses to the carrot industry despite the ability to detect seed-borne infection (Kuan et al. 1985, Umesh et al. 1996), the availability of seed treatments to eliminate seed-borne inoculum (Howard et al. 1994, Pscheidt and Ocamb 2001), and development of seed contamination thresholds for specific regions of carrot production (e.g., Umesh et al. 1998).

Empirical evidence suggests bacterial blight is more prevalent in carrot seed crops grown in central OR than in central WA, despite similarities between these two regions of seed production. The greater prevalence of bacterial leaf blight in OR compared to WA was confirmed for the 2000-2001 season when plant pathologists from California, OR, and WA toured carrot seed crops in the two states in June and July 2001. Nonetheless, the relationship between incidence/severity of bacterial leaf blight and contamination of the harvested seed has not been clarified for these semi-arid regions of seed production in the Pacific Northwest.

Objectives for this work included:

1. Identify primary sources of inoculum associated with bacterial blight of carrot seed crops in central OR and central WA.
2. Monitor development of *X. campestris* pv. *carotae* in carrot seed crops in central OR and central WA.
3. Identify environmental and cultural factors associated with the differential prevalence of bacterial blight in central OR and central WA.

Materials and Methods

Survey of Carrot Seed Crops

Ten seed-to-seed crops were selected in central Oregon and 12 seed-to-seed crops in central WA to monitor development of *X. campestris* pv. *carotae* through the 2001-2002 season under the diversity of production practices and in the range of locations/environmental conditions representative of carrot seed production in the Pacific Northwest (Table 1). Fields were sampled twice in the fall and winter (1) between 2 and 10 October in OR, and on 28 September and 5 October in WA (before fall frosts); and (2) from 6 to 8 November in OR, and 30 November or 16 January in WA (after the first fall frosts). For the second collection, snow cover in WA

prevented sampling of all fields in November. Twenty plants were collected from each field in a "W"' pattern at each sampling date. People collecting plants disinfected their hands between samples. Whole plants were sampled, placed in individual plastic bags, and stored on ice for transportation to a refrigerated facility (4-8°C).

Additional plant samples will be collected from each field in the spring (March/April) and summer (May/June, and July/August) of 2002. Two steckling-to-seed crops will be added to the survey in both OR and WA. Samples of stecklings shipped into OR and WA will be assayed for *X. campestris* pv. *carotae* to determine whether stecklings may provide inoculum for infection of seed crops.

Leaf Assays

The presence or absence of symptoms of bacterial leaf blight was recorded for each plant sampled. Plant samples (foliage only) were assayed for *X. campestris* pv. *carotae* within 2-14 hours of sampling in OR, and within 24-36 hours of sampling in WA. Plants sampled in OR were assayed at the Oregon State University-Central Oregon Agricultural Research Center (OSU-COARC); plants sampled in WA were assayed at the Washington State University (WSU) - Mount Vernon Research and Extension Unit. Fresh plant weights were measured for plants sampled in WA, and dry weights were determined after leaf extractions for plants sampled in OR (leaves oven-dried overnight at 65-70°C). The entire foliage of each plant was assayed, except for plants collected from Field WG in WA in January 2002, from which a 3-g subsample of the foliage from each plant was assayed.

Carrot leaves were assayed for *X. campestris* pv. *carotae* using the protocol described by Umesh et al. (1998), with slight modifications. In WA, foliage from each plant was cut into 1- to 4-mm pieces, placed in a 250-ml erlenmeyer flask containing 30 ml of sterile buffer (0.01 M potassium phosphate), swirled on a rotary shaker for 60 min, and the suspension concentrated 10-fold by centrifugation. The concentrate was assayed for *X. campestris* pv. *carotae* by (1) plating a dilution series (three replications of a 0.1-ml aliquot per dilution) onto XCS agar, a semi-selective medium for *X. campestris* pv. *carotae* (Williford and Schaad 1984); and (2) the polymerase chain reaction (PCR) assay developed by Umesh et al. (1996). For the PCR assay, DNA was extracted using the CTAB method (Zhang 1996) for the first set of plant samples, and the Dellaporta method (Dellaporta et al. 1983) for the second set of plant samples. In OR, foliage of each sample was cut into pieces, placed in a flask containing filtered/deionized water (50, 100, or 150 ml depending on the amount of foliage), swirled on a rotary shaker for 15 min at 250 rpm, and a dilution series prepared and plated onto XCS agar (three replications of a 0.2-ml aliquot per dilution). After incubation at 28°C for 5-10 days, colonies of *X. campestris* pv. *carotae* were counted. Representative colonies were transferred onto YDC agar (Schaad et al. 2001) for verification of colony morphology, and tested using the PCR assay (Umesh et al. 1996). PCR assays were carried out at WSU-Mount Vernon or at the University of California-Davis (personal communication, R.L. Gilbertson and R.M. Davis).

Seed Assays

Samples of stock seed for each seed crop surveyed were collected from collaborating seed companies, and assayed for *X. campestris* pv. *carotae* using a modified version of the dilution

plating protocol described by Kuan et al. (1985) and Umesh et al. (1998). Two 10-g subsamples of each stock seed lot were assayed for the bacterial pathogen. Stock seed lots from WA fields were also assayed twice by PCR. Samples of stock seed of several crops remain to be assayed. A third replication of all seed lots will be assayed. Samples of seed harvested from each field in summer 2002 will be assayed for *X. campestris* pv. *carotae* to determine the relationships between inoculum on stock seed, development of the bacterial population on plants in-season, and infection of the harvested seed.

Cultural Practices and Environmental Conditions

Production practices (irrigation system, cropping history, and pest management programs) associated with each carrot seed crop sampled will be examined in relation to development of bacterial blight and final seed-borne populations of *X. campestris* pv. *carotae*. Data on regional weather conditions (temperature, relative humidity, precipitation, frequency and timing of frosts relative to crop maturity, wind, etc.) collected from local weather stations through the 2001-2002 season will be examined relative to development of bacterial blight in the fields surveyed.

Results

The 10 direct-seeded carrot seed crops surveyed in central OR were planted between 1 August and 19 August 2001, and the 12 seed crops sampled in central WA were planted between 20 August and 5 September 2001. *Xanthomonas campestris* pv. *carotae* was detected in stock seed samples of four carrot seed crops in central WA, i.e., Fields WA (detected by PCR and dilution plating), WF (detected by dilution plating), WG (detected by PCR in one replication), and WK (detected by dilution plating) (Table 1). Populations of the pathogen in these infected seed lots ranged from 4.9×10^4 to 2.6×10^5 CFU/g seed. The pathogen was not detected in any of the stock seed samples assayed in OR (Table 1). However, 5 and 12 additional stock seed lots remain to be assayed for WA and OR, respectively.

Symptoms of bacterial leaf blight were not observed on any of the plants sampled during the first (pre-fall frost) sampling period, and *X. campestris* pv. *carotae* was isolated from only a single plant in one field (Field OE, sprinkler irrigated, at 1×10^5 CFU/g tissue) in OR during this period. During the second sampling period in central WA, *X. campestris* pv. *carotae* was found on only one plant (at 2.4×10^2 CFU/g tissue) in one field (Field WH, under furrow irrigation) (Table 2), and none of the plants sampled in WA displayed symptoms of bacterial leaf blight. In contrast, by November symptoms of bacterial leaf blight were observed in several carrot seed fields in central OR, and *X. campestris* pv. *carotae* was isolated from plants sampled from 7 of the 10 fields (Fields OA, OB, OC, OF, OG, OI, and OJ), including both sprinkler and furrow irrigated fields (Table 2). The incidence of plants in these fields that tested positive for the bacterium ranged from 10 to 45 percent, and populations of the pathogen on individual plants that tested positive ranged from 7.4×10^2 to 6.3×10^9 CFU/g dry leaf tissue.

Daily minimum and maximum temperatures, and occurrence of frosts between 1 August and 31 December 2001 were similar for central OR (measured in Madras, OR) and central WA (measured in Moses Lake, WA) (data not shown). However, 5.01 inches of total precipitation was received in Madras, OR during this 5-month period, compared to 3.36 inches of total precipitation in Moses Lake, WA. Splashed or windblown water is a primary means of spreading *X. campestris* pv. *carotae*.

Discussion

Xanthomonas campestris pv. *carotae* was more prevalent in central OR than in central WA by November 2001, approximately 3 months after planting, even though copper sprays were applied to the OR seed crops in September whereas no copper applications were made to the WA seed crops surveyed. Preliminary results of the stock seed assays suggest infected stock seed was not the primary source of inoculum in OR, as the eight stock seed lots tested were negative for *X. campestris* pv. *carotae*. Furthermore, bacterial leaf blight was not observed in WA fields that were planted with infected stock seed, even under sprinkler irrigation. There was also no evidence of greater incidence/populations of *X. campestris* pv. *carotae* in the OR fields under overhead (sprinkler) irrigation vs. fields under furrow irrigation. These results suggest seedborne inoculum may play a less significant role than other sources of inoculum (such as infested debris) in development of bacterial leaf blight in carrot seed crops in the Pacific Northwest. Additional sampling of these fields through the spring and summer of 2002, completion of the stock seed assays, and assays of the seed harvested in 2002 will provide more detailed information on the relative roles of infected stock seed and types of irrigation on: (1) development of bacterial leaf blight in-season, and (2) infection of the seed harvested from carrot seed crops in the Pacific Northwest.

To prevent pollen contamination and ensure trueness-to-type of harvested seed, carrot seed crops are separated spatially by distances ranging from ¼- to >3 miles, depending on the type or variety of the crops. This spatial separation within seasons may provide some control of bacterial leaf blight by minimizing movement of inoculum among fields. However, carrot seed crops are sometimes seeded in close (<¼ mile) proximity to mature seed crops from the previous season. The availability of fewer irrigated acres in central OR than in central WA results in more carrot seed fields in OR planted in close proximity to mature crops of the previous season than in WA. In addition, direct-seeded carrot seed crops are typically planted 3-5 weeks earlier in OR than in WA. As a result, in OR carrot seedlings are more likely to have emerged at the time nearby crops of the previous season are being harvested, potentially exposing young, susceptible plants to windblown infested debris. Work in 2002 will include atttempts to measure the distance of movement of *X. campestris* pv. *carotae* -infested dust and debris from fields during harvest.

The presence of *X. campestris* pv. *carotae* on carrot seed produced in the Pacific Northwest highlights the need to identify primary sources of inoculum leading to infections under the semi-arid conditions of this region. Identifying these sources of infection and the primary periods of infection through the biennial season will assist in development of more efficacious, regional Integrated Pest Management programs for carrot seed crops.

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Table 1. Carrot seed crops direct-seeded in central Oregon and central Washington in 2001 and surveyed for development of *Xanthomonas campestris* pv. *Carotae.*

Field	Hybrid or OP	Carrot type	Planting date (2001)	Irrigation		Stock seed assayed for *X. campestris* pv. *carotae*[a]			
						PCR assay[b]		Dilution plating (CFU/g seed)[c]	
						Replication 1	Replication 2	Replication 1	Replication 2
	Central Washington								
WA	OP	Kuroda	?	Sprinkler		+[d]	+	8.0×10^{4}	2.6×10^{5}
WB	Hybrid	Nantes	20 Aug.	Furrow	Female	-	-	-	-
					Male	-	-	-	-
WC	Hybrid	Nantes	28 Aug.	Sprinkler	Female	NT	NT	NT	NT
					Male	NT	NT	NT	NT
WD	Hybrid	Nantes/Flakkee	4 Sep.	Furrow	Female	-	-	-	-
					Male	-	-	-	-
WE	OP	Amsterdam	21 Aug.	Sprinkler		-	-	-	-
WF	OP	Chantenay	23 Aug.	Furrow		-	-	1.9×10^{5}	5.6×10^{4}
WG	OP	Flakkee	24 Aug.	Furrow		+	+	-	-
WH	OP	Chantenay	?	Furrow		-	-	-	-
WI	OP	Nantes	?	Furrow		-	-	-	-
WJ	Hybrid	Imperator	5 Sep.	Sprinkler (to emergence), drip	Female	NT	NT	NT	NT
					Male	NT	NT	NT	NT
WK	Hybrid	?	1 Sep.	Sprinkler	Female	-	-	-	-
					Male	-	-	2.2×10^{5}	4.9×10^{4}
WL	OP	Chantenay	1 Sep.	Furrow		NT	NT	NT	NT
	Central Oregon								
OA	Hybrid	Nantes	10 Aug.	Sprinkler (fall), furrow (spring)	Female	NT	NT	-	-
					Male	NT	NT	NT	NT
OB	Hybrid	Nantes	18 Aug.	Furrow	Female	NT	NT	-	-
					Male	NT	NT	NT	NT
OC	Hybrid	Nantes Amsterdam	2 Aug.	Furrow	Female	NT	NT	-	-
					Male	NT	NT	NT	NT
OD	Hybrid	Nantes	23 Aug.	Furrow	Female	NT	NT	-	-
					Male	NT	NT	NT	NT
OE	Hybrid	Nantes	14 Aug.	Sprinkler	Female	NT	NT	-	-
					Male	NT	NT	NT	NT
OF	Hybrid	Nantes	19 Aug.	Sprinkler	Female	NT	NT	-	-
					Male	NT	NT	NT	NT
OG	Hybrid	Nantes	13 Aug.	Sprinkler	Female	NT	NT	NT	NT
					Male	NT	NT	NT	NT
OH	Hybrid	Nantes	11 Aug.	Sprinkler	Female	NT	NT	-	-
					Male	NT	NT	NT	NT
OI	Hybrid	Nantes	1 Aug.	Sprinkler	Female	NT	NT	-	-
					Male	NT	NT	NT	NT

Field	Hybrid or OP	Carrot type	Planting date (2001)	Irrigation	Stock seed assayed for *X. campestris* pv. *carotae*[a]				
						PCR assay[b]		Dilution plating (CFU/g seed)[c]	
						Replication 1	Replication 2	Replication 1	Replication 2
OJ	Hybrid	Nantes	18 Aug.	Sprinkler (fall), furrow (spring	Female	NT	NT	NT	NT
		Amsterdam			Male	NT	NT	NT	NT

[a] 10-g samples of stock seed assayed for *X. campestris* pv. *carotae* as described by Umesh et al. (1998).
[b] PCR assay = polymerase chain reaction assay developed by Umesh et al. (1996).
[c] Dilution series of seed extract plated onto XCS agar (Williford and Schaad 1984), with three plates per dilution. Representative colonies transferred to YDC agar (Schaad et al. 2001) and tested by PCR assay (Umesh et al. 1996). CFU = colony forming units of *X. campestris* pv. *carotae*/g seed.
[d] +, -, NT = stock seed samples positive, negative, or not tested for *X. campestris* pv. *carotae*.

Table 2. Colony-forming-units (CFUs) of *Xanthomonas campestris* pv. *carotae* isolated from carrot plants sampled from direct-seeded carrot seed fields in central Oregon and central Washington between November 2001 and January 2002[a].

	Carrot seed fields and sampling dates (November 2001 and January 2002)									
Plant[b]	**Central Oregon (CFU/g dry weight of foliage)**									
	OA 7 November	**OB 7 November**	**OC 7 November**	**OD 7 November**	**OE 6 November**	**OF 6 November**	**OG 6 November**	**OH 7 November**	**OI 8 November**	**OJ 7 November**
1	0	0	0	0	0	0	0	0	7.2×10^4	0
2	0	0	1.3×10^6	0	0	0	2.2×10^7	0	0	0
3	0	0	0	0	0	5.7×10^4	0	0	8.1×10^5	0
4	0	0	2.7×10^7	0	0	0	0	0	0	0
5	1.1×10^3	0	4.8×10^5	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	4.5×10^8	0
7	0	0	0	0	0	0	1.3×10^8	0	0	0
8	5.6×10^3	0	0	0	0	0	1.6×10^6	0	1.0×10^5	0
9	0	0	3.9×10^3	0	0	0	5.7×10^6	0	0	0
10	0	0	5.3×10^8	0	0	2.0×10^5	6.3×10^9	0	9.1×10^5	8.0×10^7
11	0	5.0×10^9	0	0	0	0	0	0	5.1×10^7	4.2×10^6
12	0	0	0	0	0	0	7.5×10^8	0	0	0
13	0	2.0×10^5	0	0	0	0	6.3×10^5	0	0	0
14	0	5.9×10^5	0	0	0	0	0	0	9.4×10^6	1.2×10^8
15	0	2.7×10^7	0	0	0	0	0	0	0	1.3×10^6
16	0	0	0	0	0	0	0	0	0	0
17	0	1.1×10^5	0	0	0	0	0	0	2.4×10^4	1.7×10^9
18	1.8×10^3	0	1.6×10^9	0	0	0	0	0	7.4×10^5	1.7×10^5
19	0	0	0	0	0	0	0	0	0	0
20	7.4×10^2	0	0	0	0	0	0	0	0	0
Mean[c]	2.3×10^3	1.0×10^9	3.6×10^8	0	0	1.3×10^5	1.0×10^9	0	5.7×10^7	3.2×10^8

	Central Washington (CFU/g fresh weight of foliage)											
Plant	**WA 16 January**	**WB 30 November**	**WC 30 November**	**WD 30 November**	**WE 16 January**	**WF 16 January**	**WG 16 January**	**WH 30 November**	**WI 30 November**	**WJ Not sampled**	**WK 16 January**	**WL 16 January**
1	0	0	0	0	0	0	0	0	0	-	0	0
2	0	0	0	0	0	0	0	0	0	-	0	0
3	0	0	0	0	0	0	0	0	0	-	0	0
4	0	0	0	0	0	0	0	0	0	-	0	0
5	0	0	0	0	0	0	0	0	0	-	0	0
6	0	0	0	0	0	0	0	0	0	-	0	0
7	0	0	0	0	0	0	0	0	0	-	0	0
8	0	0	0	0	0	0	0	0	0	-	0	0
9	0	0	0	0	0	0	0	0	0	-	0	0
10	0	0	0	0	0	0	0	0	0	-	0	0
11	0	0	0	0	0	0	0	0	0	-	0	0
12	0	0	0	0	0	0	0	0	0	-	0	0
13	0	0	0	0	0	0	0	0	0	-	0	0
14	0	0	0	0	0	0	0	0	0	-	0	0
15	0	0	0	0	0	0	0	0	0	-	0	0
16	0	0	0	0	0	0	0	0	0	-	0	0
17	0	0	0	0	0	0	0	0	0	-	0	0
18	0	0	0	0	0	0	0	0	0	-	0	0
19	0	0	0	0	0	0	0	0	0	-	0	0
20	0	0	0	0	0	0	0	2.4×10^2	0	-	0	0
Mean[c]	0	0	0	0	0	0	0	2.4×10^2	0	-	0	0

[a] Foliage of each plant assayed for *X. campestris* pv. *carotae* using the protocol described by Umesh et al. (1998).
[b] Twenty plants sampled in a "W"pattern from each field.
[c] Mean = average CFU/g foliage for those plants that tested positive for *X. campestris pv. carotae*