BACTERIAL BLIGHT OF CARROT SEED CROPS: IDENTIFICATION OF SOURCES OF INOCULUM

Lindsey duToit, Fred Crowe, Mike Derie, and Rhonda Bafus

Introduction

Carrot seed crops in central Washington and central Oregon produce approximately 75% of the U.S. carrot seed (Thomas et al., 1997) on 2,000 to 3,000 acres per state (Pelter, 2001). As a seedborne disease, bacterial blight, caused by Xanthomonas campestris pv. carotae, is a 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 can occur on foliage, stems, umbels, and seed of carrot or carrot seed crops. Leaf symptoms start as small, irregular, chlorotic areas, expanding to irregular water-soaked lesions. Lesions often appear a greasy green-black color, but may become tan as they dry and are surrounded by an irregular yellow halo. Dark brown, linear lesions form on stems and petioles. A gummy bacterial exudate may develop. If flowers on a seed plant are infected early in development, entire umbels may be blighted. If umbel infection occurs later, the umbels are typically partially blighted. Seed yield (quality and quantity) may be affected, and seed may be infected internally or contaminated on the seed surface. Infection of seed by X. campestris pv. carotae may reduce germination, resulting in losses to seed growers if germination is <85%. Seed companies, in turn, face expenses associated with treating infected seed lots, and seed lots may be rejected by importing markets.

Cultivated carrot is the only known host of X. campestris pv. carotae. Being seedborne, the pathogen can survive on or in carrot seed and spread (long or short distances) through the movement of infested or infected seed. The bacterium persists in infested carrot residues in the soil for up to a year. Bacteria are dispersed by splashing water and insects. The presence of water is necessary for infection to occur, with the pathogen reproducing most rapidly under warm (77–86°F) and wet conditions. Symptoms appear within 10 to 12 days of inoculation, and the disease develops rapidly under warm and wet conditions. Planting healthy seed or treated seed is an important first step in management of bacterial blight. Management recommendations include a 2- to 3-year crop rotation, and incorporating residues into the soil promptly after harvest to reduce survival of the pathogen. Overhead irrigation creates favorable conditions for development of bacterial leaf blight and increases dispersal of the bacterium relative to furrow or drip irrigation. Seed can be disinfested by soaking in hot water (122°F) for 30 minutes. Some resistance to bacterial leaf blight is available in commercial cultivars.

Bacterial blight continues to cause losses to the carrot industry despite the ability to detect seedborne infection (Kuan et al., 1985, Umesh et al., 1996), the availability of seed treatments to eliminate seedborne 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 leaf blight is more prevalent in carrot seed crops grown in central Oregon than in central Washington, despite similarities between these two regions of seed production. This was confirmed in the 2000-2001 season when plant pathologists from California, Oregon, and Washington toured carrot seed crops in the two states in June/July 2001. The nature of the relationship between incidence and severity of bacterial leaf blight and contamination of the harvested seed remains to be clarified for the semi-arid regions...
of seed production in the Pacific Northwest. The objectives of this project funded by the California Fresh Carrot Advisory Board (CFCAB) are to:

1. Identify primary sources of inoculum associated with bacterial blight of carrot seed crops in central Oregon and central Washington.
3. Identify environmental and cultural factors associated with the differential prevalence of bacterial blight in central Oregon and central Washington.

This paper describes results of research from the 2001-2002 season. Additional funding has been received from the CFCAB to repeat the project in 2002-2003. Preliminary results have been presented (du Toit and Crowe, 2002, du Toit et al., 2002).

**Materials and Methods**

**2001-2002 Survey of Carrot Seed Crops**

Ten and twelve direct-seeded crops were selected in central Oregon and central Washington, respectively, 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). The first letter of the code for each field sampled in Oregon and Washington begins with an “O” and an “W”, respectively. Fields were sampled twice in the fall/winter: 1) between 2 and 10 October in Oregon, and on 28 September and 5 October in Washington (before fall frosts); and 2) from 6 to 8 November in Oregon, and 30 November or 16 January in Washington (after the first fall frosts). Snow cover in Washington prevented sampling of all fields in November. At each sampling date, 20 plants were collected from each field in a “W”-pattern. Individuals collecting the plants disinfected their hands between samples using 70% ethyl alcohol. Whole plants were sampled, placed in individual plastic bags, and stored on ice for transportation to a refrigerated facility (4 to 8°C).

Steckling carrot seed crops in each of Fields OK, OL, OM, ON, WN, and WO were added to the survey in spring 2002 (Table 1). Twenty plants were sampled from the direct-seeded and steckling fields between 1 and 9 April in Oregon, and between 28 March and 11 April in Washington. For the steckling crops, plants were sampled directly from the crates in which the stecklings had been shipped from California, and for the direct-seeded crops, plants were sampled as described above. The direct-seeded crops in Fields OC, OD, WA, and WH were dropped from the survey prior to this sampling period as the crops were not needed by the seed companies and were plowed under by the growers. The crop in Field OK was dropped from the survey after samples were collected in April.

Twenty plant samples were collected again from each field between 4 and 10 June in Oregon, and on 3 or 10 June in Washington. Samples were collected from each field as described above. However, in Washington, samples were collected from the perimeter of each open pollinated crop as the crops were too dense to walk through. Individuals collecting samples walked 10-20ft into the crop from the edge of the field at each sampling location. For hybrid seed crops in Washington, samples were collected approximately 10ft within the crop from the location of the wheel-lines, irrigation pipes, or alleys between the rows of male and female parents. In both
states, whole plants were sampled or, where plants were >2.5 ft tall, samples of leaves (3-4 per plant), stems (2-3 stem sections per plant, including nodes), and umbels (3 or 4 per plant) were sampled. At each sampling location, plant tissue(s) showing symptoms suggestive of bacterial blight were included in the sample. Field OJ was dropped from the survey after samples were collected in June. A final set of 20 plants was sampled from each field between 15 and 24 August in Oregon, and between 22 and 30 July in Washington, as described for samples collected in June. The seed crops were swathed, windrowed, and harvested between mid-August and late-September 2002.

**Leaf, Stem, and Umbel Assays**

The presence or absence of symptoms of bacterial blight was recorded for each plant sampled. Plant samples (foliage, stems, and umbels) were assayed for *X. campestris* pv. *carotae* within 2-14 hours of sampling in Oregon, and within 24-36 hours of sampling in Washington. Plants sampled in Oregon were assayed at the Oregon State University - Central Oregon Agricultural Research Center (OSU-COARC); plants sampled in Washington were assayed at the Washington State University - Mount Vernon Research and Extension Unit (WSU – Mount Vernon REU). Fresh plant weights were measured for plants sampled in Washington in the fall/winter of 2001, and dry weights were determined after leaf extractions for all plants sampled in Oregon and for plants sampled in the spring/summer of 2002 in Washington. Samples were oven-dried for 12-36 hours at 65-70°C. For the first two sets of plant samples, the entire foliage of each plant sampled was assayed, except for plants collected from Field WG in January 2002, from which a subsample of 3 g of the foliage of each plant was assayed. For the third, fourth, and fifth sampling periods, a subsample of up to 50 g of leaves, umbels, and stems per 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 Washington, foliage from each plant was cut into 1 to 4-mm pieces, placed in a 250-ml Erlenmeyer flask containing 30 ml sterile buffer (0.01 M potassium phosphate), swirled on a rotary shaker for 60 minutes, 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 (October 2001), and the Dellaporta method (Dellaporta et al., 1983) for the second set of plant samples (November 2001/January 2002). Direct PCR assays of the leaf/stem/umbel extracts were not carried out for the third, fourth, and fifth sets of samples as the plating assay proved more sensitive than the PCR assay and enabled the bacterial population to be quantified (vs. the qualitative PCR assay). For the third, fourth, and fifth sets of samples, the volume of buffer used in the extraction procedure was increased to 100-ml per sample and the washes carried out in 500-ml Erlenmeyer flasks. The centrifugation step was also eliminated from the extraction procedure for the fourth and fifth sets of samples. In Oregon, 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 minutes at 250 rpm, and a dilution series plated onto XCS agar (3 replications of a 0.2-ml aliquot per dilution). Leaf extractions for the third, fourth, and fifth collections in Oregon were carried out using sterile buffer (0.01 M potassium phosphate) instead of sterile deionized water, and the duration samples
were placed on a rotary shaker was increased to 60 minutes. After incubation at 28°C for 5-10 days, colonies typical of *X. campestris* pv. *carotae* were counted. Representative colonies from each field 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 done at the WSU-Mount Vernon REU, or at the University of California-Davis (by R.L. Gilbertson and R.M. Davis).

**Seed Assays**
Samples of stock seed for each seed crop surveyed (where available) 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 or three 10-g subsamples of each stock seed lot were assayed for the bacterial pathogen. Stock seed lots from Washington fields were also assayed twice by PCR. Samples of stock seed of several crops were not provided. Samples of seed harvested from each field in summer 2002 were assayed for *X. campestris* pv. *carotae* to determine the relationship between populations of the pathogen on stock seed, development of the bacterial population on plants in-season, and infection of the harvested seed.

**Pathogenicity Tests**
Representative bacterial colonies from Washington samples (plant and seed samples) that resembled *X. campestris* pv. *carotae* on XCS and YDC agar, and that tested positive for *X. campestris* pv. *carotae* by the PCR assay, were also tested for pathogenicity on carrot seedlings in the greenhouse. A suspension of bacterial cells (approximately 10⁶ cfu/ml) of each isolate was atomized onto 5 seedlings. Inoculated plants were placed in plastic bags for 3 days in the greenhouse to create a humid environment, and monitored for up to 6 weeks for symptoms of bacterial blight. A known pathogenic isolate of *X. campestris* pv. *carotae* was included in each pathogenicity test. Similar pathogenicity tests were carried out for representative bacterial isolates obtained from Oregon samples and which resembled *X. campestris* pv. *carotae* on XCS and YDC agar.

**Sampling of Dust and Debris During Threshing of Carrot Seed Crops**
An Anderson spore sampler was used to determine whether clouds of dust and debris generated during combining/threshing of carrot seed crops could generate a source of airborne *X. campestris* pv. *carotae* for infection of newly emerged carrot seedlings in neighboring direct-seeded crops planted for the subsequent cropping season. The spore sampler was powered by a generator and placed on the back of a pickup truck. The truck was driven at distances ranging from 25ft to approximately 1 mile behind or downwind of a combine/thresher during threshing of the seed crops in each of four locations in central Oregon in September 2002 (Table 3). Two Petri plates with XCS agar medium were placed in the spore sampler to trap airborne cells of *X. campestris* pv. *carotae* at each distance, and the air was sampled from 1 second to 15 minutes. A hand-held anemometer was used to record the average wind speed and direction each time the spore sampler was operated.

**Cultural Practices and Environmental Conditions**
Information on production practices (irrigation system, cropping history, and pest management programs) associated with each carrot seed crop sampled was collected to examine in
relationship to development of bacterial blight and final seedborne populations of *X. campestris pv. carotae*. Data on regional weather conditions (minimum, maximum, and average daily temperatures; total daily precipitation; frequency and timing of frosts relative to crop maturity; and wind speed) were collected from local weather stations through the 2001-2002 season to compare with development of bacterial blight in the fields surveyed.

**Results**

The ten direct-seeded carrot seed crops surveyed in central Oregon were planted between 1 August and 19 August 2001, and the 12 direct-seeded crops sampled in central Washington were planted 3-5 weeks later, between 20 August and 5 September 2001 (Table 1). Symptoms of bacterial blight were not observed on any of the plants sampled during the first (pre-fall frost) sampling period, and *X. campestris pv. carotae* was not detected on any of the plants sampled in Washington (Figs. 1 and 3); however, the pathogen was isolated from one plant in one field in Oregon (Field OE, sprinkler irrigated) during this period (Fig. 2), at a population of $1 \times 10^5$ colony forming units (CFU)/g tissue (Fig. 4). During the second sampling period, *X. campestris pv. carotae* was found on one plant in one field in Washington (Field WH, furrow irrigated) at $2.4 \times 10^2$ CFU/g tissue (Fig. 3), and none of the plants displayed symptoms of bacterial blight. Although symptoms of bacterial blight were not observed in any of the fields sampled in central Oregon in November, *X. campestris pv. carotae* was detected in 8 of the 10 crops sampled in this state after the first fall frosts (Fig. 2), including both sprinkler and furrow irrigated fields (Table 1). The incidence of plants that tested positive for *X. campestris pv. carotae* in these crops ranged from 2/20 (10%) to 9/20 (45%), and the mean population of *X. campestris pv. carotae* per plant that tested positive ranged from $1.7 \times 10^3$ to $4.6 \times 10^8$ CFU/g tissue (Fig. 4).

By spring (March/April) of 2002, *X. campestris pv. carotae* was detected in 3 of the 12 seed crops sampled in Washington, i.e., from 1/20 plants from the direct-seeded crops in each of Fields WD and WG, and from 3/20 plants from the steckling crop in Field WN (Fig. 1). The plants in Field WN had been picked directly out of the crates in which the stecklings had been shipped from the Imperial Valley, California, indicating the stecklings were probably a source of inoculum in that crop. The population of *X. campestris pv. carotae* detected in Washington during this sampling period ranged from $2.5 \times 10^7$ to $5.2 \times 10^8$ CFU/g tissue (Fig. 3), although none of the plants showed obvious symptoms of bacterial blight. In Oregon, *X. campestris pv. carotae* was detected in 9 of the 12 crops sampled in April (all the direct-seeded crops and one of four steckling crops), at an incidence ranging from 2/20 (10%) to 11/20 plants (55%) (Fig. 2), with a mean population on individual plants ranging from $1.8 \times 10^4$ to $8.9 \times 10^8$ CFU/g tissue (Fig. 4). Symptoms of bacterial blight were evident in several crops in Oregon.

By early June of 2002, *X. campestris pv. carotae* was detected in 5 of the 12 seed crops sampled in Washington, at an incidence ranging from 1/20 (5%) to 5/20 (25%) plants per field, and a mean population ranging from $1.4 \times 10^4$ to $4.1 \times 10^7$ CFU/g tissue (Fig. 3). The pathogen was not detected in either of the steckling crops in Washington (Fields WN and WO). As in April 2002, none of the plants sampled showed definitive symptoms of bacterial blight. In Oregon, only the crop in Field ON tested negative for *X. campestris pv. carotae* in June (Fig. 2). The incidence of the pathogen detected in the remaining 11 crops ranged from 2/20 (10%) to 18/20
Symptoms of bacterial blight were evident in most fields sampled in Oregon in June. The incidence of carrot seed plants on which *X. campestris* pv. *carotae* was detected in central Washington increased from early June to late July 2002, when the pathogen was found in 11 of the 12 crops sampled, at an incidence ranging from 2/20 plants (10% in Field WN) to 20/20 plants (100% in Field WD) (Fig. 1), and a mean population ranging from $1.9 \times 10^3$ (Field WJ) to $1.8 \times 10^8$ CFU/g tissue (Field WL) (Fig. 3). In August 2002, *X. campestris* pv. *carotae* was detected in all 10 crops sampled in central Oregon (Fig. 2), at an incidence ranging from 3/20 plants (15% in Field OM) to 20/20 plants (100% in Fields OB and OF) (Fig. 2), and a mean population ranging from $1.2 \times 10^4$ (Field OM) to $1.0 \times 10^9$ CFU/g tissue (Field OG) (Fig. 4).

Although fewer steckling crops than direct-seeded crops were sampled in each state, the mean incidence of plants that tested positive for *X. campestris* pv. *carotae* was usually greater in the direct-seeded crops than in the steckling crops. The average incidence of plants that tested positive for *X. campestris* pv. *carotae* by midsummer (July/August 2002) was 17.0 and 11.7 out of 20 plants for the direct-seeded crops in Oregon and Washington, respectively, and 7.0 and 1.0 out of 20 plants for the steckling crops in Oregon and Washington, respectively (Table 2).

**Seed Assays**

*Xanthomonas campestris* pv. *carotae* was detected in stock seed samples of five carrot seed crops in central Washington, i.e., Fields WA (detected by PCR and dilution plating), WE and WF (by dilution plating), WG (by PCR in one replication), and WK (by dilution plating) (Table 1). The mean population of *X. campestris* pv. *carotae* in these infected seed lots ranged from $1.9 \times 10^2$ to $2.6 \times 10^5$ CFU/g seed (Table 1, Fig. 5). The pathogen was not detected in the 7 stock seed samples assayed in Oregon (Table 1). However, 19 of the 26 stock seed lots (male and female parents for 13 hybrid seed crops) for fields sampled in Oregon were not available to assay for *X. campestris* pv. *carotae*. Similarly, stock seed lots for the steckling crops surveyed in Washington were not assayed for *X. campestris* pv. *carotae*, and neither was the stock seed for Field WL.

At the time this report was prepared, one replication of assays of harvested seed had been completed for 8 of the 12 crops surveyed in Washington, with *X. campestris* pv. *carotae* detected in 6 of the 8 harvested seed lots (Fig. 5). The population of the pathogen detected in these seed lots ranged from $4.2 \times 10^5$ CFU/g seed (Field WG) to $6.3 \times 10^7$ CFU/g seed (Field CD). The pathogen was not detected in seed harvested from the steckling crop in Field WN, even though the pathogen was found on stecklings sampled directly out of the shipping crates in April. Harvested seed from only 1 of the 10 direct-seeded crops (Field WJ) tested negative for *X. campestris* pv. *carotae*. Samples of harvested seed were only available for 6 of the 14 crops surveyed through to harvest in Oregon (Fig. 6). Results of a single assay of 10g of seed from these 6 seed lots showed *X. campestris* pv. *carotae* to be present in 4 of the seed lots, at populations ranging from $7.1 \times 10^4$ CFU/g seed (Field OA) to $9.9 \times 10^7$ CFU/g seed (Field OH).

**Pathogenicity Tests**

All bacterial isolates that formed colonies characteristic of *X. campestris* pv. *carotae* on XCS and YDC agar and that tested positive for this pathovar using the PCR assay proved pathogenic when
inoculated onto carrot seedlings in the greenhouse. A few isolates that resembled *X. campestris* pv. *carotae* on XCS agar did not test positive for this pathovar using the PCR assay. These isolates were not pathogenic when inoculated onto carrot seedlings, supporting the value of the PCR assay for identification of isolates of *X. campestris* pv. *carotae*.

**Sampling of Dust and Debris During Threshing of Carrot Seed Crops**

*Xanthomonas campestris* pv. *carotae* was detected in the clouds of dust/debris generated downwind of the seed threshers in three of the four locations sampled (Table 3). The population of *X. campestris* pv. *carotae* detected varied depending on the wind direction, wind speed, and location (ranging from 0.02 to 33.70 CFU/ft³ air). At Location 2, the population of bacterium detected decreased with increasing distance from the thresher, and the pathogen was not detected further than 200 ft from the thresher. However, at Location 4, the pathogen was detected approximately one mile from the location of the seed crop being threshed. This sampling location was within 1.5-5 miles of about 10 carrot seed crops being harvested at approximately the same time, so the few CFUs of *X. campestris* pv. *carotae* detected at this site could have originated from any of a number of seed crops being harvested.

**Cultural Practices and Environmental Conditions**

Daily minimum and maximum temperatures and occurrence of frosts between 1 August and 31 December 2001 were similar for central Oregon (measured in Madras, OR) and central Washington (measured in Moses Lake, WA) (data not shown). Heat units [measured as the sum (average daily temperature – 50°F)] received in Madras during this period totaled 1,005°F vs. 1,180°F received in Moses Lake. Total precipitation received in Madras during this five-month period was 4.10 inches, compared to 3.36 inches in Moses Lake. Weather records for the two regions from 1 January to 31 December 2002 showed Madras received a total of 5.7 inches precipitation vs. 7.0 inches for Moses Lake. Although the number of days in which the average temperature dropped below freezing was similar for the two regions in 2002 (44 days in Madras and 43 days in Moses Lake), the total heat units received in Madras during 2002 (1,973°F) was 28% less than that received in Moses Lake during the same period (2,529°F). In fall 2001, two to three copper applications were made in most of the carrot seed crops surveyed in central Oregon; fall copper applications were not made in any of the seed crops surveyed in central Washington. Data are being collated on copper applications made in spring/summer 2002 in the crops surveyed.

**Discussion**

*Xanthomonas campestris* pv. *carotae* was more prevalent in carrot seed crops in central Oregon than in central Washington throughout the 2001-2002 season. This difference in prevalence was apparent as early as the fall of 2001, approximately three months after planting, even though copper sprays were applied to the Oregon seed crops in September but not to any of the seed crops surveyed in Washington that fall. The limited results of the stock seed assays suggest infected stock seed was not a primary source of inoculum in Oregon, as the eight stock seed lots tested were negative for *X. campestris* pv. *carotae*. Furthermore, bacterial leaf blight was not observed in crops in Washington that were planted with infected stock seed, even under sprinkler irrigation. There was also no evidence of greater incidence or populations of *X. campestris* pv. *carotae* in the Oregon crops grown using overhead (sprinkler) irrigation vs. crops grown using
furrow irrigation. These results suggest seedborne inoculum may play a less significant role than other sources of inoculum (e.g., infested debris) in development of bacterial leaf blight in carrot seed crops in the Pacific Northwest.

The population of *X. campestris* pv. *carotae* increased earlier and more rapidly in seed crops in central Oregon than in central Washington. In both regions, a majority of the plants that tested positive for the pathogen appeared to have been colonized epiphytically, as symptoms of bacterial blight were not evident until early summer in Oregon and mid- to late summer in Washington (if at all). Weather conditions were very similar between the two regions, although central Washington received 28% more heat units than central Oregon in 2002. Weather data will be examined in greater detail relative to the increase in populations of *X. campestris* pv. *carotae* detected in seed crops surveyed in the two regions.

The incidence and population of *X. campestris* pv. *carotae* was typically lower in steckling crops than in direct-seeded crops. However, the pathogen was isolated from stecklings sampled directly out of the shipping crates for two of the crops surveyed (OL and WN), indicating that infected stecklings could be a source of inoculum for seed production in the Pacific Northwest. This would be of particular concern when steckling crops are grown under overhead irrigation, as supported by the observation that the infrequent epidemics of bacterial blight in carrot seed crops in central Washington occur primarily when infected stecklings are planted under overhead irrigation (G.Q. Pelter, personal observation). Detection of the bacterial pathogen on stecklings sampled directly from the shipping crates emphasizes the importance of producing stecklings in regions where bacterial blight is not endemic, and/or treating stecklings for *X. campestris* pv. *carotae* (e.g., dipping stecklings in a chlorine solution prior to cold storage or transplanting).

The population of *X. campestris* pv. *carotae* detected on harvested seed samples appeared to be correlated with the population/incidence of the pathogen detected on plants sampled late in the season (July 2002). For example, the seed harvested from Fields WJ and WN tested negative for *X. campestris* pv. *carotae*, and these two fields also had the lowest incidence of plants from which *X. campestris* pv. *carotae* was isolated in July 2002 and the lowest population of the pathogen on those plants from which the pathogen was detected. Conversely, the seed harvested from Fields WD, WK, and WL had the highest populations of *X. campestris* pv. *carotae*, and the crops in these fields had the highest incidences of plants from which the bacterium was isolated in July 2002. Preliminary data on harvested seed samples assayed from 6 of the Oregon crops support this relationship. Seed harvested from Field OA had the lowest population of *X. campestris* pv. *carotae* of the six seed samples assayed, and this field had the lowest incidence and population of the pathogen on plants sampled in August 2002. There did not appear to be a strong correlation between the population of *X. campestris* pv. *carotae* detected on stock seed and the population detected on harvested seed, although the lack of stock seed samples for all of the crops surveyed deterred the ability to evaluate this relationship more effectively.

To prevent pollen contamination and ensure trueness-to-type of harvested seed, carrot seed crops are separated spatially by distances ranging from ¼-mile to >3 miles, depending on the type/variety of the seed crops. This spatial separation within seasons may provide some control of bacterial blight by minimizing movement of inoculum among neighboring fields. However, carrot seed crops are sometimes seeded in close proximity (<¼ mile) to mature seed crops from
the previous season. The availability of fewer irrigated acres in central Oregon than in central Washington results in more carrot seed fields in Oregon being planted in close proximity to mature crops of the previous season compared with Washington. In addition, direct-seeded crops are typically planted three to five weeks earlier in Oregon than in Washington. As a result of the longer “green-bridge” effect in Oregon, 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. We were able to detect airborne *X. campestris* pv. *carotae* up to a mile downwind of seed crops being harvested/threshed.

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 the sources of infection and the primary periods of infection through the biennial season will assist in development of more efficacious regional IPM programs for bacterial blight on carrot seed crops.

**Literature Cited**

Acknowledgements

We thank the California Fresh Carrot Advisory Board for funding this project; Mike Davis, Bob Gilbertson, Joe Nunez, and Shawn Meng for information on the bacterial assays; the Columbia Basin Vegetable Seed Association and carrot seed companies in Washington and Oregon for information on carrot seed crop production and for access to carrot seed fields; Gary Pelter for regular assistance collecting samples in Washington; and Barbara Holmes, Raina Spence, Kari Berglund, Sarah Lloyd, Sam Sampson, and Katie Baber for assistance in processing the plant and seed samples.
Table 1. Carrot seed crops surveyed in central Oregon and central Washington in 2001-2002 for development of *Xanthomonas campestris* pv. *carotae*.

<table>
<thead>
<tr>
<th>Field</th>
<th>Hybrid or OP</th>
<th>Carrot type</th>
<th>Planting date</th>
<th>Irrigation</th>
<th>Stock seed assayed for <em>X. campestris</em> pv. <em>carotae</em>a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>PCR assayb Dilution plating (CFU/g seed)c</td>
</tr>
<tr>
<td>Central Washington 2001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>+d  +  8.0 x 10^4  2.6 x 10^5  4.3 x 10^5  2.6 x 10^5</td>
</tr>
<tr>
<td>WA OP</td>
<td>Kuroda</td>
<td>Sprinkler</td>
<td>20 Aug.</td>
<td>Female</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td>WB Hybrid</td>
<td>Nantes</td>
<td>28 Aug.</td>
<td>Sprinkler</td>
<td>Female</td>
<td>NT  NT  -  -  -  -  In process</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>NT  NT  -  -  -  -  In process</td>
</tr>
<tr>
<td>WC Hybrid</td>
<td>Nantes/Flakkee</td>
<td>4 Sep.</td>
<td>Furrow</td>
<td>Female</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td>WE OP</td>
<td>Amsterdam</td>
<td>21 Aug.</td>
<td>Sprinkler</td>
<td>-  -</td>
<td>4.3 x 10^5  3.3 x 10^1  1.0 x 10^2  1.9 x 10^2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.6 x 10^5  -  -  -  -</td>
</tr>
<tr>
<td>WF OP</td>
<td>Chantenay</td>
<td>24 Aug.</td>
<td>Furrow</td>
<td>+  +</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td>WG Hybrid</td>
<td>Flakkee</td>
<td>?</td>
<td>Furrow</td>
<td>-  -</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td>WH OP</td>
<td>Chantenay</td>
<td>5 Sep.</td>
<td>Sprinkler</td>
<td>Female</td>
<td>NT  NT  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>NT  NT  -  -  -  -  -</td>
</tr>
<tr>
<td>WI OP</td>
<td>Nantes</td>
<td>2 Sep.</td>
<td>Sprinkler</td>
<td>Female</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td>WJ Hybrid</td>
<td>Imperator</td>
<td>1 Sep.</td>
<td>Sprinkler</td>
<td>Female</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td>WK Hybrid</td>
<td></td>
<td>1 Sep.</td>
<td>Sprinkler (fall),</td>
<td>Female</td>
<td>NT  NT  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>drip (spring)</td>
<td>Male</td>
<td>NT  NT  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td>WL OP</td>
<td>Chantenay</td>
<td>1 Sep.</td>
<td>Furrow</td>
<td>-  -</td>
<td>-  -  -  -  -  -  -</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.2 x 10^5  4.93 x 10^4  2.6 x 10^4  9.8 x 10^4</td>
</tr>
</tbody>
</table>

Central Washington 2002

| WN Hybrid  | Nantes/Amsterdam | Mar/Apr. | Furrow | Female | NT  NT  NT  NT  NT  NT  NT  NT |
|            |                  |          |        | Male   | NT  NT  NT  NT  NT  NT  NT  NT |
| WO Hybrid  | Imperator        | Mar/Apr. | Sprinkler | Female | NT  NT  NT  NT  NT  NT  NT  NT |
|            |                  |          |        | Male   | NT  NT  NT  NT  NT  NT  NT  NT |

Central Oregon 2001

<p>| OA Hybrid  | Nantes         | 10 Aug.   | Sprinkler | Female | NT  NT  NT  NT  NT  NT  NT  NT |
|            |                |          | (fall), furrow (spring) | Male   | NT  NT  NT  NT  NT  NT  NT  NT |
| OB Hybrid  | Nantes         | 18 Aug.   | Furrow    | Female | NT  NT  NT  NT  NT  NT  NT  NT |
| OC Hybrid  | Nantes/Amsterdam| 2 Aug.    | Furrow    | Female | NT  NT  NT  NT  NT  NT  NT  NT |
| OD Hybrid  | Nantes         | 23 Aug.   | Furrow    | Female | NT  NT  NT  NT  NT  NT  NT  NT |
| OE Hybrid  | Nantes         | 14 Aug.   | Sprinkler | Female | NT  NT  NT  NT  NT  NT  NT  NT |</p>
<table>
<thead>
<tr>
<th>Field</th>
<th>Hybrid or OP</th>
<th>Carrot type</th>
<th>Planting date</th>
<th>Irrigation</th>
<th>PCR assay&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Dilution plating (CFU/g seed)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>OF</td>
<td>Hybrid Nantes</td>
<td>19 Aug.</td>
<td>Sprinkler</td>
<td>Male NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>OG</td>
<td>Hybrid Nantes</td>
<td>13 Aug.</td>
<td>Sprinkler</td>
<td>Male NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>OH</td>
<td>Hybrid Nantes</td>
<td>11 Aug.</td>
<td>Sprinkler</td>
<td>Male NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>OI</td>
<td>Hybrid Nantes</td>
<td>1 Aug.</td>
<td>Sprinkler</td>
<td>Male NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>OJ</td>
<td>Hybrid Nantes</td>
<td>1 Aug.</td>
<td>Sprinkler</td>
<td>Male NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>18 Aug. Sprinkler (fall), furrow (spring)</td>
<td>Male NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Female NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Central Oregon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OL</td>
<td>Hybrid Emperor</td>
<td>Mar/Apr.</td>
<td>Sprinkler</td>
<td>Female NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>OM</td>
<td>Hybrid Emperor</td>
<td>Mar/Apr.</td>
<td>Sprinkler</td>
<td>Female NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>ON</td>
<td>Hybrid Nantes</td>
<td>Mar/Apr.</td>
<td>Furrow</td>
<td>Female NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Male NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

<sup>a</sup> Ten-g samples of stock seed assayed for *X. campestris* pv. *carotae* as described by Umesh et al. (1998).

<sup>b</sup> PCR assay = polymerase chain reaction assay developed by Umesh et al. (1996).

<sup>c</sup> 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.

<sup>d</sup> +, -, NT = stock seed samples positive, negative, or not tested for *X. campestris* pv. *carotae*.
Table 2. Mean incidence of plants on which *Xanthomonas campestris* pv. *carotae* was detected in direct-seeded and steckling carrot seed crops sampled through the 2001-2002 season in central Oregon and central Washington.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oregon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct-seeded</td>
<td>0.1</td>
<td>4.5</td>
<td>5.9</td>
<td>10.5</td>
<td>17.0</td>
</tr>
<tr>
<td>Steckling</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
<td>1.3</td>
<td>7.0</td>
</tr>
<tr>
<td>Washington</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct-seeded</td>
<td>0.0</td>
<td>0.1</td>
<td>0.2</td>
<td>1.1</td>
<td>11.7</td>
</tr>
<tr>
<td>Steckling</td>
<td>-</td>
<td>-</td>
<td>1.5</td>
<td>0.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* a The number of direct-seeded crops and steckling crops sampled in central Oregon ranged from 7 to 10 and 3 to 4, respectively.

* b The number of direct-seeded crops sampled in central Washington ranged from 10 to 12. Two steckling crops were sampled in Washington.


<table>
<thead>
<tr>
<th>Location</th>
<th>Distance from seed thresher</th>
<th>No. of samples</th>
<th>Mean CFU/ft³ air</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100’</td>
<td>10</td>
<td>13.30</td>
<td>18.70</td>
</tr>
<tr>
<td></td>
<td>150’</td>
<td>7</td>
<td>33.70</td>
<td>43.20</td>
</tr>
<tr>
<td></td>
<td>250’</td>
<td>3</td>
<td>1.80</td>
<td>1.30</td>
</tr>
<tr>
<td>2</td>
<td>25’</td>
<td>4</td>
<td>15.00</td>
<td>30.00</td>
</tr>
<tr>
<td></td>
<td>100’</td>
<td>12</td>
<td>10.40</td>
<td>34.50</td>
</tr>
<tr>
<td></td>
<td>150’</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>200’</td>
<td>8</td>
<td>1.20</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>300’</td>
<td>1</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>800’</td>
<td>3</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>900’</td>
<td>8</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>4b</td>
<td>~1 mile</td>
<td>5</td>
<td>0.02</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* a Two Petri dishes with XCS agar medium were placed in an Anderson spore sampler for each sampling period. At each location the spore sampler was placed on the back of a pickup truck driven at the stated distances (approximately downwind) from a carrot seed thresher in operation. The duration of each sampling period ranged from 1 second to 15 minutes.

* b Location 4 was within 1.5 to 5.0 miles of about 10 other carrot seed crops being harvested during approximately the same one- to two-week period.
Fig. 1. Incidence of plants from which *Xanthomonas campestris* pv. *carotae* was isolated in 14 carrot seed crops (12 direct-seeded and 2 steckling) sampled through 2001/02 in Washington.
**Fig 2.** Incidence of plants from which *Xanthomonas campestris* pv. *carotae* was isolated in 14 carrot seed crops (10 direct-seeded and 4 steckling) sampled through 2001/02 in Oregon.

**Fig 3.** Mean population of *Xanthomonas campestris* pv. *carotae* detected on plants from which the pathogen was isolated in 14 carrot seed crops (12 direct-seeded and 2 steckling) sampled in 2001/02 in Washington.
Fig. 4. Mean population of *Xanthomonas campestris* pv. *carotae* detected on plants from which the bacterial pathogen was isolated in 14 carrot seed crops (10 direct-seeded and 4 steckling) sampled in 2001/02 in Oregon.

Fig. 5. Population of *Xanthomonas campestris* pv. *carotae* detected in stock seed and/or harvested seed samples from 14 carrot seed crops grown in Washington in 2001/02. The
crops in Fields WA to WL were direct-seeded, and in Fields WN and WO were grown from stecklings. Missing bars indicate seed samples were not available.

**Fig. 6.** Population of Xanthomonas campestris pv. carotae detected in stock seed and/or harvested seed samples of carrot seed crops grown in Oregon in 2001/02. The crops in Fields OA to OJ were direct-seeded, and in Fields OK to ON were grown from stecklings. Missing bars indicate seed samples were not available.