SCREENING CARROT GERMPLASM FOR RESISTANCE TO *XANTHOMONAS HORTORUM* PV. *CAROTAE*

By

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To the Faculty of Washington State University:

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SCREENING CARROT GERMPLASM FOR RESISTANCE TO

XANTHOMONAS HORTORUM* PV. *CAROTAE

Abstract

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Xanthomonas hortorum pv. *carotae* causes bacterial blight of carrot and is readily seedborne. Genetic resistance is limited in commercial cultivars, and there has been little public research on screening for resistance. Carrot plant introduction (PI) lines (n = 66) from the United States Department of Agriculture (USDA) National Plant Germplasm System, two inbred lines from the USDA Agricultural Research Service, and 17 commercial cultivars were screened for resistance to *X. hortorum* pv. *carotae* in a greenhouse in 2012. Evaluations were based on the percentage of foliage that developed symptoms as well as population (CFU/g dry foliage) of the pathogen on the foliage detected on a semi-selective agar medium. Severity of foliar blight ranged from 0 to 50% (mean ± SE of 8.8 ± 0.4%), and the pathogen population ranged from 1.38 x 10^4 to 3.28 x 10^{11} CFU/g dry foliage (8.16 x 10^9 ± 1.07 x 10^9 CFU/g) for individual plants. Eight putative resistant PI lines and five highly susceptible PI lines selected from the 2012 screening were evaluated again in 2013 along with an additional 2 PI lines, 12 cultivars, 2 inbreds, and 16 carrot wild relatives. Severity of foliar blight 6 wpi ranged from 0 to 90% (11.8 ± 0.4%), and the pathogen population ranged from 4.90 x 10^4 to 1.30 x 10^{11} CFU/g dry foliage (1.00 x 10^{10} ± 5.29 x 10^8 CFU/g) for individual plants. In 2012 and 2013, Spearman's correlation coefficients between bacterial populations and symptoms were highly significant (r =

0.5183 and 0.6162, respectively, at $P < 0.0001$). PIs 418967, 432905, and 432906 were the most resistant based on pathogen population detected. There was little resistance displayed by the carrot wild relatives. Resistance from the selected PIs will be integrated into commercially-acceptable carrot inbreds (USDA ARS inbred lines A2566 and A0493) for release to public and private carrot breeding programs. Additionally, assays of 25 seed/PI revealed the presence of *Alternaria radicina* on 56.1%, *A. carotiincultae* on 1.5%, *A. dauci* on 51.5%, and *A. petroselini* on 1.5% of the seed lots received from the USDA NPGS, highlighting the need for treatment of carrot PI seed.

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Literature review

1.1. *Daucus carota.*

Significance. Carrot (*Daucus carota* L. var. *sativus*) is produced worldwide for its taproot (Rubatzky et al., 1999). In 2009, over 28 million metric tons of carrots were produced globally [United States Department of Agriculture (USDA) Economic Research Service (ERS), 2012]. China, Russia, and the United States, the three top carrot producing countries in the world, yielded > 10 million, 1.5 million, and 1.3 million metric tons of carrots in 2009, respectively (USDA ERS, 2012). Globally, the area farmed to carrots totaled more than 1.2 million ha. Carrot growers in the China, Russia, and United States grew 527,306, 67,500, and 33,156 ha of carrots in 2009, respectively (USDA ERS, 2012). From a productivity standpoint, Iceland yielded the most carrots per unit area at 80 metric tons/ha, whereas the United States, Russia, and China yielded 39.3, 22.5, and 19.1 metric tons/ha, respectively.

The total value of the United States carrot root crop is approximately $694 million a year, and fresh market carrots account for nearly 95% of the total carrot value [USDA National Agricultural Statistics Service (NASS), 2014]. The area of land planted to carrots in the United States was > 34,000 ha in 2013 with California leading production at almost 71% of the nation's 1.35 million metric tons of carrots (USDA NASS, 2014). Other prominent carrot growing states include Washington, Michigan, Wisconsin, and Texas. In 2013, Washington was ranked second nationally for total carrot production (USDA NASS, 2014). California produces most of the fresh market carrots (82%), while Washington led in production for the processing carrot market with 115,830 metric tons (36%) in 2013 (USDA NASS, 2014). Prior to 2000, Washington produced 2 to 4% of the United States' fresh market carrots. In the 1990s, carrot production in

Washington generated anywhere from $15 to $38 million of revenue (USDA ERS, 2011). The primary types of carrot grown in Washington are Imperator and Chantenay (Sorensen, 2000). After 2000, fresh market statistical information provided by the USDA NASS was discontinued for Washington alone, and production statistics in that state were merged with other states' production estimates (USDA ERS, 2011).

History. Prior to the 10th century AD, the primary part of the carrot that was used by people was thought to be the seed (Andrews, 1949). Carrot seed was used as a medicine and many cultures still consider carrot seed as having medicinal characteristics (Rubatzky et al., 1999). The first report of carrot roots consumed as a food is from Iran around the 10th century AD (Rubatzky et al., 1999). Subsequent reports of carrot root consumption were in Spain, Italy, and Northern Europe in the 12th, 13th, and 14th centuries, respectively (Rubatzky et al., 1999). The first carrot roots cultivated and consumed in these countries are thought to have been purple or yellow. It was not until the 1600s that orange and white colored carrots were cultivated in Europe. Paintings from the 17th century suggest when orange carrots may have been cultivated in Holland, while orange-types were not seen in Germany, France, and England until the 18th and 19th centuries (Banga, 1963). However, Stolarczyk and Janick (2011) showed paintings of orange-rooted carrots from the 6th century. Despite these paintings, the ancestor of the majority of modern orange rooted carrot cultivars are thought to have been selected for in Holland (Banga, 1963; Stolarczyk and Janick, 2011) Relative to other crops around the world, the carrot root crop was domesticated recently.

Today, carrots grown and consumed in eastern countries are characterized by red to purple and yellow root colors, while carrots grown and consumed in western countries are orange, yellow, red, or white-rooted (Rubatzky et al., 1999). There are also differences in leaf

characteristics between carrots consumed in eastern and western countries (Rubatzky et al., 1999). Carrots grown and consumed in eastern countries typically have pubescent leaves, causing the foliage to have a blue tint, whereas carrots grown and consumed in western countries lack pubescence on the leaves with a greener tint to the foliage (Rubatzky et al., 1999). Despite these differences, carrots consumed in both eastern and western countries belong to *D. carota* var. *sativus*.

Selections in the 18th century for carotene pigmented roots may have led to a "genetic bottleneck" that has limited relative variability within the germplasm of orange carrots cultivars (Pierre and Bayer, 1991). A genetic bottleneck is characterized by low genetic variability within a population caused by the elimination of genotypes through selection, climatic change, or mass destruction (Pierre and Bayer, 1991). The relative lack of variation in the western orange carrot may have limited breeders' ability to select for unique traits, specifically, genetic resistance to diseases and pests, although, there has not been much breeding activity in carrot, historically, compared to other crops. Germplasm resource centers may be used by breeders to introduce genetic variation from wild carrots (*D. carota* var. *carota*) or carrots consumed in eastern countries into the relatively homogenous orange carrot (Rubatzky et al., 1999). The USDA Agricultural Research Service (ARS) North Central Regional Plant Introduction Station (NCRPIS) has 1,370 accessions of the genus *Daucus,* including 31 taxa from 64 countries (Reitsma and Clarck, 2013). Public and private breeding programs have been selecting from this collection for genetic improvements to traits such as root vigor, seed production, consumer quality, and resistance to pests and pathogens (Rubatzky et al., 1999). Carrot disease resistance breeding has focused primarily on fungal and nematode pathogens, with some success; however, not much effort has gone into selection for resistance to bacterial pathogens (Stein and

Nothnagel, 1995). Fungal and nematode pathogens of carrot tend to outweigh bacterial pathogens in number and significance, which may account for the limited focus on bacterial pathogens in breeding carrots for resistance to diseases (Boiteux and Simon, 2002). However, in some regions, bacterial pathogens can be a significant issue (e.g., du Toit et al., 2005; Godfrey and Marshall, 2002).

Botany and physiology. *D. carota* is a dicotyledonous plant in the Apiaceae (Umbelliferae) (Porter, 1967). Plants in the Umbelliferae are characterized by the formation of a compound umbel, clusters of umblets made up of several pedicels attached to flowers that form a structure similar in shape to an umbrella (Porter, 1967). Carrots are biennial, i.e., they produce seed in the second growing season after vernalization. The first year's growth is vegetative, when the foliage forms a rosette with a near non-existent stem. The sexual stage produced in the second season is characterized by the elongation of a floral stem, with production and subsequent branching of the primary (king), secondary (queen), tertiary, and lower order umbels (Rubatzky et al., 1999). Flowers produced on the umbel are perfect, with male and female reproductive parts. Each fruit has two mericarps which are shed as two true seeds (Rubatzky et al., 1999).

Ideally, the carrot root crop should be grown in full sun, in a climate with moderate temperatures ranging from 15 to 21°C, and low humidity to minimize disease pressure (Rubatzky et al., 1999). Typically, carrots go through a process called vernalization between the first and second seasons of growth in order to flower. Vernalization requires the carrot plant be exposed to temperatures between 0 and 5°C for 8 to 10 weeks in order to flower the next growing season (Simon, 2004). Vernalization causes the flat, vegetative meristem to become conical, and capable of stem elongation and inflorescence production. Most carrot cultivars grown and consumed in eastern countries tend to flower earlier than the carrot cultivars grown

and consumed in western countries, which must go through an extended vernalization in order to flower (Rubatzky et al., 1999).

Breeding. Since the carrot flower is difficult to emasculate, seed production in carrots was accomplished through open pollination prior to the discovery of male sterile germplasm (Rubatzky et al., 1999). Male sterility allows a breeder or seed producer to perform cross-pollination without the arduous job of removing the stamens from the flower by hand (Poehlman, 1979). Male sterility in carrots opened a doorway to hybridization that would have otherwise been impossible on a commercial scale (Stein and Nothnagel, 1995). Male sterility in carrots is accomplished exclusively through cytoplasmic male sterility (CMS) (Stein and Nothnagel, 1995). Carrot CMS is derived from the cytoplasm of the mother (seed producing) plant, as opposed to genetic male sterility which is controlled solely by nuclear genes (Acquaah, 2007). The use of CMS in carrot breeding is also dependent on nuclear genes that can restore fertility or maintain sterility in the carrot flower. There are two separate types of CMS in carrots: brown anther and petaloid (Thompson, 1966; Welch and Grimball, 1947). Brown anther CMS forms as a result of abortion of the pollen during the formation of the anther (Welch and Grimball, 1947). Petaloid CMS is initiated during formation of the flower, when petals form in place of the stamen (Thompson, 1966). The resulting flower has an additional whorl of petals, one more than in a normal, non-CMS, flower. The most commonly used carrot CMS in the United States is petaloid CMS because tertiary or secondary umbels in brown anther CMS carrots reportedly tend to produce male fertile flowers (Rubatzky et al., 1999). Brown anther is the predominant CMS found in Europe and Asia since there are no reports of the flowers of brown anther CMS lines developed in those regions having the male fertility problems that are seen in the United States brown anther CMS lines (Rubatzky et al., 1999).

In addition to commercial hybrid production, carrot CMS can be useful for breeders who wish to make test crosses between genetically distinct carrot plants (Allard, 1960). A test cross is a cross between a plant that is homozygous recessive for a trait and a plant expressing the dominant phenotype but with unknown genotype (Acquaah, 2007). Test crosses can also be used for genetic linkage analysis with F1 plants that have known genotypes. When a CMS carrot plant is crossed with a male fertile carrot plant, seed harvested from the CMS male sterile plant will be hybrid.

Commercial carrot seed production. Carrot seed production requires two growing seasons in order to produce a seed crop (Rubatzky et al., 1999). The high value biennial seed crop is more complex and expensive to grow compared with the annual root crop. Hybrid carrot seed crop growers have the additional difficulty of getting the parent lines to "nick," i.e., getting both parent lines to flower at the same time so maximum pollination may occur between the male and female plants (Rubatzky et al., 1999). Carrot seed crops are grown primarily in two ways: 1) from seed-to-seed, and 2) from vernalized root-to-seed. Both methods require the carrot roots go through a vernalization process.

The seed-to-seed method of carrot seed production involves planting stock seed in late summer, letting the roots go through vernalization in the same field, and harvesting the seed in late summer of the next year (Rubatzky et al., 1999). A cold period of <15°C for at least 10 weeks is needed in order to vernalize the roots in the ground (Simon, 2004). In some areas of carrot seed production there can be a risk of roots freezing when growing seed-to-seed carrots, i.e., the climate must be cold enough to vernalize the roots but not so cold that roots freeze in the ground (Simon, 2004). Additionally, in the semi-arid, inland Pacific Northwest, United States, the primary region of carrot seed production in the United States, seed-to-seed carrot crops run a

high risk of frost-heaving and desiccation from cold winds when there is not adequate snow cover to protect the carrots in the winter (Lindsey du Toit, *personal communication*; Hart and Butler, 2004).

The root-to-seed method of carrot seed production involves growing out the root (steckling) in a different field or location from where the seed crop will be grown eventually, harvesting the roots after the roots achieve an optimal size, vernalizing the roots in cold storage, planting the vernalized stecklings in spring in a region where seed production is optimal, and harvesting the seed in late summer of that second season (Rubatzky et al., 1999). This method is preferable in areas where the climate or cultural practices favor the development of important diseases, where the risk of winter kill is great, or for specific carrot parent lines that are more prone to winter kill. Growers avoid planting stecklings in the same field where carrot root crops are produced to prevent a "green bridge" for moving pests and pathogens between root and seed crops. Such green bridges allow pests and pathogens of carrots to survive and infest or infect nearby seed crops for the following season (du Toit et al., 2005).

The optimal climate for carrot seed production includes mild winters (if growing the crop from seed-to-seed), dry and warm summers to reduce disease pressure, and a dry harvest season to maintain high quality and pathogen-free status of harvested seed (Simon, 2004). In order to avoid unwanted cross-pollination, carrot seed crops should not be grown where there is an abundance of wild carrot (Queen Anne's lace, *D. carota* var. *carota*). Carrot seed is grown primarily in the United States, Europe, South Africa, New Zealand, and, more recently, in Chile (Lindsey du Toit, *personal communication*). Oregon, Washington, Idaho, and Northern California are the main regions of carrot seed production in the United States. The inland Pacific Northwest (Idaho, Oregon, and Washington) produces 85% of the United States' carrot seed with

approximately 375 kg/ha on an average of approximately 3,100 ha from 2011 to 2012 (Butler and Simmons, 2012; Hart and Butler, 2004; Mike Talkington, *personal communication*; Washington State Commission on Pesticide Registration, 2011). Washington growers produce carrot seed on approximately 1,012 ha with a value of $1.5 to $5 million (Washington State Commission on Pesticide Registration, 2011). From 2007 to 2012, Idaho growers averaged 360 ha in carrot seed production, 90% of which was hybrid carrots (Mike Talkington, *personal communication*). These areas produce the majority of carrot seed in the United States because of the climate, irrigation water, and absence of wild carrot needed for successful carrot seed production (Hart and Butler, 2004).

1.2. *Xanthomonas hortorum* pv. *carotae*.

Carrot foliar diseases. The American Phytopathological Society (APS) lists 7 bacterial, 49 fungal, 5 nematode, 10 viral, and 1 phytoplasma carrot pathogens (Strandberg, 2000). Of the 49 fungal carrot pathogens listed, 3 are causes of major foliar diseases: *Alternaria dauci* which causes Alternaria leaf blight, *Cercospora carotae* which causes Cercospora leaf spot, and *Erysiphe heraclei*, *Leveillula taurica*, and *L. lanuginosa* which cause powdery mildew (Aegerter, 2002; Rubatzky et al., 1999). Of the seven bacterial carrot pathogens listed, one causes a major foliar disease: *Xanthomonas hortorum* pv. *carotae* which causes bacterial blight of carrot (Rubatzky et al., 1999). Symptoms of Alternaria leaf blight, Cercospora leaf spot, and bacterial leaf blight on carrot include necrotic lesions on the foliage. Often, in the field, these foliar diseases can be misidentified because of similarities in the range of symptoms (Boedo et al., 2010). Symptoms of carrot leaf blights may be expressed differently depending on the host genotype (Krämer et al., 2009). In Washington, diseases are the primary concern in carrot grower pest management programs (Sorensen, 2000).

Xanthomonas. *X. hortorum* pv. *carotae* belongs to a diverse group of bacteria called the Xanthomonads (Vauterin et al., 1995). The genus *Xanthomonas* is characterized by the yellow pigments produced when the bacteria are grown on media containing sugar. These yellow pigments are known as xanthomonadins, which are brominated aryl-polyenes, and are the primary ingredient in xanthan gum produced by *Xanthomonas* spp., which is also used as a thickening agent in foods (Andrewes et al., 1976; Chun, 2002; Kado, 2010). Originally, species of *Xanthomonas* were named and differentiated based on the phenotype of host specificity (Vauterin et al., 1995). This approach yielded an overabundance of *Xanthomonas* species. In 1980, a committee appointed by the International Society for Plant Pathology (ISPP) reclassified phytopathogenic *Xanthomonas* species into pathovars of the species *X. campestris* (Dye et al., 1980). However, the more than 140 pathovars of *X. campestris* were thought not to be based on genomic relationships among all the pathovars of this species but, rather, the pathovars were based on the relationships with the host plants. In 1995, Vauterin et al. (1995) reclassified the genus *Xanthomonas*, particularly *X. campestris* pathovars, based on phenotypic, genotypic, and chemotaxonomic relationships among species and pathovars. *X. campestris* pv. *carotae,* a pathogen of *D. carota*, was reclassified as *X. hortorum* pv. *carotae* as a result of DNA hybridization (Palleroni et al., 1993; Vauterin et al., 1995).

Bacterial blight of carrot. Bacterial blight caused by *X. hortorum* pv. *carotae* is a furtive disease of carrot often going undetected in carrot seed crops while infesting the seed. The pathogen is disseminated by seed, movement of infested debris remaining in carrot fields after harvest, splashing water from irrigation or precipitation, insects, machinery, and people moving through infected canopies of carrot crops (Gilbertson, 2002). The host range of *X. hortorum* pv. *carotae* is limited primarily to carrots; however, there are reports of pathogenicity on other

Umbelliferous crops. Poplawsky et al. (2004) reported symptoms caused by *X. hortorum* pv. *carotae* on celery, coriander, dill, fennel, lovage, parsley, and parsnip plants inoculated in greenhouse trials. The infections on these other Umbelliferous crops may not occur readily in natural environments (Poplawsky et al., 2004). The presence of moisture on leaves is required for initiation of infection, and splashing water is one of the most effective means of dispersal of the pathogen, e.g., from overhead irrigation (Gilbertson, 2002). Development of bacterial blight occurs most rapidly at 25 to 30°C, but can develop at greater and lesser temperatures (Pfleger et al., 1974). *X. hortorum* pv. *carotae* can persist at fairly high populations on carrot foliage without the plants showing any symptoms of bacterial blight. Generally, disease symptoms only start developing when epiphytic populations of *X. hortorum* pv. *carotae* reach 10^5 to 10^6 colony forming units (CFUs) per gram of carrot foliage (Gilbertson, 2002). As a consequence, infected, symptomless plants often go undetected and, when symptoms finally develop, it is too late for effective management with protectant bactericide treatments (du Toit et al., 2005).

Seed can be a significant source of primary inoculum, but other sources of inoculum also can play an important role in development of bacterial blight (du Toit et al., 2005). Umesh et al. (1998) found that seed contamination levels of 10^4 to 10^5 CFU/g seed were needed in order for a yield loss to be documented in sprinkler-irrigated, fresh market carrot root crop trials in California. This threshold for seed borne inoculum is used by the carrot seed industry as a standard when testing seed lots, and seed companies routinely treat carrot seed lots when contamination levels are above this threshold (du Toit et al., *in press*). Aerosolized particles generated during threshing of carrot seed crops can be a source of inoculum for carrot seed crops during the first season of the carrot seed crop biennial lifecycle (du Toit et al., 2005). Aerosolized particles infested with *X. hortorum* pv. *carotae* can blow ≤ 1.6 km downwind of the

carrot seed crop being harvested (du Toit et al., 2005). In addition to contaminated seed and aerosolized carrot seed crop particles, infection of carrot crops by *X. hortorum* pv. *carotae* is often exacerbated by extended periods of leaf wetness and water splash when crops are grown using overhead irrigation (du Toit et al., 2005; Gilbertson, 2002). This is one of the primary reasons for widespread adoption of drip irrigation in carrot seed production in the Pacific Northwest (Weber et al., 2004).

In root crops, symptoms caused by *X. hortorum* pv. *carotae* can be similar to those initially associated with Alternaria leaf blight (Pryor and Strandberg, 2002). Symptoms appear as small, angular spots on foliage, which eventually turn into greasy-looking, brown lesions that become tan and brittle in dry conditions (Gilbertson, 2002). The lesions commonly arise on the 'V'-shaped area between leaflet lobes (Gilbertson, 2002). In carrot seed crops, partial or whole umbels can become blighted but even asymptomatic umbels can produce infected seeds, hindering the production of pathogen free seed from infected umbels (du Toit et al., 2005). Establishment of *X. hortorum* pv. *carotae* in a seed field most likely ensures the infection of seed produced in that field, thus perpetuating the disease cycle.

Detection of *Xanthomonas hortorum* pv. *carotae.* There are multiple methods for detecting *X. hortorum* pv. *carotae* on carrot foliage and seed. Seed may be assayed using a seed wash and dilution plating technique [International Seed Trade Association (ISTA), 2013e]. The seed wash method starts with weighing multiple replicates of seed from each seed lot to be tested (du Toit et al., *in review*). Each replicate subsample is soaked in saline or phosphate buffer overnight (16 to 18 hours) at 4 to 7°C or at room temperature (20 to 24°C) for 2 h. This soaking step results in the bacteria moving from the seed into the solution without stimulating inordinate bacterial population growth, to facilitate quantifying the level of seed infection. One to two

drops of Tween 20 is added as a detergent to break the surface tension between the seed and buffer solution (Kuan et al., 1985). The flask with the seed suspension is then placed on a shaker for 5 minutes at 250 rpm. The seed suspension is then poured through cheesecloth as a filter to remove the seed. If bacterial populations are expected to be low in the seed wash a concentration-by-centrifugation step may be added to enable more accurate bacterial population counts than without centrifugation. A 10-fold dilution series (to at least 10^{-3}) of the seed wash is then plated subsequently in 100 µL aliquots onto XCS agar, on which each aliquot is spread using a bent glass rod, to determine the CFU/g seed assayed (du Toit et al., 2005; du Toit et al., *in review*). XCS is a semi-selective agar medium for the detection of *X. hortorum* pv. *carotae* (Williford and Schaad, 1984). Other semi-selective agar media that may be used for detection of *X. hortorum* pv. *carotae* are TMB, KM-1, MD5A, and MKM (du Toit et al., *in review*; ISTA, 2013e).

Similarly, carrot foliage can be assayed for *X. hortorum* pv. *carotae* (e.g. du Toit et al., 2005). Foliage removed from the plant is cut into small pieces (~5 mm^2), soaked in a phosphate buffer, and may be concentrated using a centrifuge if bacterial populations are expected to be low. A dilution series of the foliar wash can either be plated in 10 to 100 µL aliquots on XCS agar medium, and/or a polymerase chain reaction (PCR) assay can be run on the suspension using *X. hortorum* pv. *carotae*-specific primers (Meng et al., 2004). Foliage and other tissues (e.g. umbels and stalks) can be assayed using these methods (du Toit et al., 2005). If whole plants cannot be assayed because the volume of above-ground plant material is too great, then samples of representative tissues of the whole plant can be sampled, e.g., for carrot plants in the reproductive growth phase (du Toit et al., 2005).

The PCR assay developed by Meng et al. (2004) for confirmation of the bacterial blight pathogen on foliage and seed uses 3S PCR primers for detection of *X. hortorum* pv. *carotae*. Bacterial suspensions are prepared from suspect colonies isolated from carrot seed or foliage assays. Alternatively, a concentrated seed or foliar wash can be assayed directly to detect the presence of *X. hortorum* pv. *carotae*. DNA is extracted from the sample, and the PCR assay is run. The PCR products are then visualized using gel electrophoresis. The presence of *X. hortorum* pv. *carotae* is indicated by a ~350 bp amplicon in the gel. This assay can detect *X. hortorum* pv. *carotae* in carrot foliage or seed infected at levels $>10^2$ CFU/g leaf tissue or seed (Meng et al., 2004). However, the PCR assay does not discriminate between DNA of live vs. dead (viable vs. non-viable) *X. hortorum* pv. *carotae*, and the assay is not quantitative (Meng et al., 2004; du Toit et al., *in review*). A real-time PCR assay and a loop-mediated isothermal amplification (LAMP) assay were developed in combination with the use of a DNA-binding dye, propidium monoazide (PMA) by Temple et al. (2013) to quantify levels of DNA of viable *X. hortorum* pv. *carotae*. PMA is a DNA-intercalating dye, when combined with appropriate molecular assays for DNA or RNA of a target organism, enables the detection of DNA or RNA from only viable target cells (Nocker et al., 2006). The PMA real-time PCR assay and PMA LAMP assays of Temple et al. (2013) can be used to detect and quantify DNA of viable *X. hortorum* pv. *carotae* in pure culture, and from infested carrot seed at levels as low as 10^2 to 10^3 CFU/g seed.

***Xanthomonas hortorum* pv. *carotae* management strategies.** One of the primary means of managing bacterial blight in carrots is planting pathogen-free seed (Gilbertson, 2002). If detected in a seed lot, *X. hortorum* pv. *carotae* can be eradicated from most seed lots with a hot water treatment in which the seed is agitated in water heated to 52°C for 25 minutes, followed

rapidly by cold water washes of the seed to remove the heat, and drying of the seed to minimize adverse effects of the treatment on seed vigor and shelf-life (Gilbertson, 2002). Seed companies are often reluctant to use hot water seed treatment, however, because of the cost, turnover, amounts of seed to treat, and potential adverse effects that hot water treatment can have on seed. If temperatures are too high during treatment, seed germination can be compromised or the hot water treatment may lower the shelf life and vigor of the treated seed, and if temperatures are too low, the pathogen may not be controlled efficiently (du Toit et al., 2005). Despite the potential negative effects that hot water treatment can have on carrot seed, Strandberg and White (1989), Pryor et al. (1994), and Nega et al. (2003) showed that hot water treatment has a great benefit of controlling seed borne pathogens, e.g., *A. dauci*, *A. radicina*, and *X. hortorum* pv. *carotae*.

Growers can implement certain cultural practices in order to minimize development of bacterial blight in carrot crops. Sprinkler irrigation is a primary means of spread of *X. hortorum* pv. *carotae* and, if possible, overhead irrigation should be avoided (Gilbertson, 2002; Weber et al., 2004). If overhead irrigation cannot be avoided, completing irrigation cycles early in the daytime is ideal so that foliage has time to dry (Pelter and du Toit, 2003). Drip irrigation is being utilized increasingly in carrot seed crops in central Oregon to reduce water usage, increase yields, and decrease disease pressure from pathogens like *X. hortorum* pv. *carotae* (Weber et al., 2004). Incorporation of crop debris into the soil soon after harvest facilitates faster decomposition than leaving residues on the soil surface, helping to eliminate some of the *X. hortorum* pv. *carotae* associated with carrot debris as the pathogen is not soilborne (Gilbertson, 2002; Pelter and du Toit, 2003). Growers can also use spatial and temporal isolation of seed-to-seed crops to manage *X. hortorum* pv. *carotae* infection from aerosolized particles generated during the threshing of carrot seed crops. Planting stecklings in place of seed-to-seed carrot

crops is a temporal isolation management option to minimize carrot seed crop infection by aerosolized particles. However, planting stecklings for root-to-seed crops does not ensure that the seed crop will be free of *X. hortorum* pv. *carotae*. If stecklings are suspected of being infected, the roots can be dipped in a chlorine solution to manage any source of primary inoculum (Pelter and du Toit, 2003).

A 2- to 3-year crop rotation (or longer) should be practiced to ensure that populations of *X. hortorum* pv. *carotae* do not build up from annual cropping of carrot (Gilbertson, 2002). du Toit et al. (2005) showed that, despite applications of bactericides, use of furrow or drip irrigation, hot water seed treatment, and incorporation of crop debris into the soil to prevent bacterial blight inoculum build up, carrot seed growers in the Pacific Northwest regularly experience infection by *X. hortorum* pv. *carotae* in their seed crops. However, infection levels were shown to be significantly less as a result of these preventative disease management practices and, in a few of the carrot seed fields tested over two biennial seasons, *X. hortorum* pv. *carotae* was not detected on the crop or on the harvested seed (du Toit et al., 2005).

An important preventative disease management practice is protective spray applications of copper prior to infection by *X. hortorum* pv. *carotae* (du Toit and Derie, 2008a). Applications of copper bactericides are used to manage bacterial diseases in various crops (e.g., Gracia-Garza et al., 2002; Gent and Schwartz, 2005; Mmbaga and Nnodu, 2006). Copper bactericides can significantly reduce *X. hortorum* pv. *carotae* populations during the vegetative and reproductive phases of carrot growth and on harvested seed when compared with other treatments (du Toit and Derie., 2005; du Toit et al., 2006; du Toit and Derie., 2008a and 2008b). Copper hydroxide (CuOH) is the most effective of the copper bactericides tested although other copper formulations (e.g., copper ammonium complex) have been shown to significantly reduce *X.*

hortorum pv. *carotae* on carrot foliage (Crowe and Simmons, 2005; du Toit and Derie., 2005; du Toit et al., 2006; du Toit and Derie., 2008a and 2008b). ManKocide (Dupont, Wilmington, DE), a mixture of the fungicide mancozeb and the bactericide CuOH (Kocide), was most effective among all treatments tested in reducing bacterial populations on carrot foliage and harvested seed; CuOH control of *X. hortorum* pv. *carotae* is enhanced by the zinc in mancozeb (du Toit and Derie., 2005; du Toit et al., 2006; du Toit and Derie., 2008a and 2008b). However, although bacterial populations can be reduced using coppers, complete control of the pathogen usually is not accomplished with bactericides (e.g., du Toit and Derie., 2008a and 2008b). du Toit and Derie (2008a) showed that, as inoculum populations became greater (from 10^2 to 10^6 CFU/ml inoculum) , preinoculation applications of Mankocide became less effective and bacterial populations recovered from foliage increased (from 0.00 to 7.58 $\log_{10}$ CFU/g dry foliage). Monitoring carrot seed fields throughout the growing season for establishment of *X. hortorum* pv. *carotae* and development of bacterial blight is a proactive measure that enables a grower to control the pathogen before it becomes established in the field, thereby increasing the probability of producing seed with minimal levels of *X. hortorum* pv. *carotae* (Gilbertson, 2002).

In addition to hot water seed treatment and cultural practices for bacterial blight management, the seed industry is looking for additional ways to control bacterial blight. Breeding efforts aimed at resistance to bacterial blight have been limited, but Pfleger et al. (1974) showed that there may be genetic differences in resistance to *X. hortorum* pv. *carotae* among carrot cultivars (Boiteux and Simon, 2002). When the genome of *X. hortorum* pv. *carotae* was sequenced, orthologs of type III effector genes were identified (Kimbrell et al., 2012). When examined *in planta*, *X. hortorum* pv. *carotae* was shown to elicit a gene-mediated hypersensitive response (Kimbrell et al., 2012). The same type III effector genes found in *X.*

hortorum pv. *carotae* by Kimbrell et al. (2012) trigger hypersensitive resistance responses in tomato and pepper to *X. campestris* pv. *vesicatoria* (Minsavage et al., 1990; Schulte and Bonas, 1992; Stall et al., 2009). Leite et al. (1994) showed that homologs of *hrp* genes of *X. campestris* pv. *vesicatoria* are present in *X. hortorum* pv. *carotae*. *Hrp* genes are a type III effector gene and associated with disease symptoms produced on susceptible host plants and hypersensitive reactions in resistant host plants inoculated with these pathogens (Willis et al., 1991). The presence of various pathogenicity and effector genes in *X. hortorum* pv. *carotae* indicates there may be more effective levels of resistance to *X. hortorum* pv. *carotae* within carrot germplasm than resistance currently available in commercial cultivars, or that there simply has not been much effort yet on breeding for resistance to this pathogen.

If genetic resistance is bred into parent lines of commercial hybrid cultivars, the benefits could be utilized by both seed growers and root growers. With genetic resistance to bacterial blight, hot water and disinfectant treatments may not be needed as regularly to meet the current seed threshold of 10^4 to 10^5 CFU *X. hortorum* pv. *carotae* / g seed that is followed by the seed industry in the United States (Umesh et al., 1998). Genetic resistance to *X. hortorum* pv. *carotae* could reduce overall costs of production for growers. Furthermore, Mexico recently enacted a zero tolerance policy for *X. hortorum* pv. *carotae* on carrot seed (Lindsey du Toit, *personal communication*), and if such policies are enacted more globally, the carrot seed industry could benefit from access to more resistant cultivars than currently available to combat bacterial blight.

1.3. *Alternaria* spp. in carrot.

Carrot black rot. Black rot of carrot is caused by *Alternaria radicina* and was first described in New York in 1922 (Meier et al., 1922). *A. radicina* reportedly also infects parsley, celery, caraway, dill, fennel, and parsnip, but the primary host is carrot (Farrar et al., 2004;

Pryor, 2002a). Mature conidia are ellipsoid or ovoid, brown, and typically 42 to 50 μm x 18 to 25 μm (Simmons, 1995). Conidia are beakless and solitary or in clusters of two to three per conidiophore. Conidiophores may grow on aerial mycelium or directly from mycelium on the substrate, and are 20 to 80 μm long when isolates are grown on potato carrot agar (PCA) medium (Simmons, 1995; Simmons and Roberts, 1993). *A. radicina* does not have a known sexual phase (Farrar et al., 2004).

Black rot symptoms typically appear as a soft, black decay at the crown of the carrot plant, but also can appear as dry, black, sunken lesions on the root surface (Farrar et al., 2004). Petioles and foliage may also become infected, resulting in leaf blight similar to that caused by *Alternaria dauci* (Farrar et al., 2004; Meier et al., 1922). Planting seeds infected or infested with spores and/or mycelium of *A. radicina* can result in pre- and post-emergence damping-off (Pryor, 2002b). *A. radicina* is especially problematic on carrot roots in cold storage as the pathogen can cause entire infected roots to decay, and can spread among roots (Farrar et al., 2004). Without early identification and adequate management practices, black rot can result in devastating economic losses during carrot root or seed production. Infected or infested seed, infested debris from previous carrot crops, and spores spread by wind or splashing water can all be sources of *A. radicina* inoculum (Farrar et al., 2004; Maude and Shuring, 1972).

Morphological and phylogenetic studies have led to separation of isolates of *A. radicina* from those of *A. carotiincultae*, a species similar in appearance, pathogenesis, and ancestry to *A. radicina* (Lawrence et al., 2012; Park et al., 2008; Simmons, 1995). Conidia of *A. carotiincultae* differ from *A. radicina* conidia in that spores of the former are typically more ellipsoid or ovoid than *A. radicina*, and obovoid conidia are observed rarely (Simmons, 1995). Dimensions of *A. carotiincultae* conidia grown on PCA after 10 days are 40 to 60 μm x 20 to 23 μm for ovoid

conidia, and 65 to 80 μm x 15 to 20 μm for ellipsoid conidia (Simmons, 1995). *A. carotiincultae* causes black rot symptoms similar to those caused by *A. radicina*, and studies indicate that *A. carotiincultae* is equally, if not more, virulent on carrot roots than *A. radicina* (Pryor and Gilbertson, 2002). However, *A. carotiincultae* is not commonly found in carrot crops and is rarely problematic for carrot growers (Pryor and Gilbertson, 2002).

Alternaria leaf blight of carrot. First described by Kuhn in Germany in 1855, Alternaria leaf blight of carrot is caused by *A. dauci* and can be found in all carrot producing areas of the world (Pryor and Strandberg, 2002; Simmons, 1995). *A. dauci* has been found infecting wild and cultivated carrot, and has been reported to cause a disease on celery and parsley (Farrar et al. 2004; Simmons, 1995). Conidia of *A. dauci* are typically dark brown, obclavate, 80 to 100 μm x 15 to 24 μm, and have 8 to 11 transepta and 1 to 3 longitudinal septa (Simmons, 1995). The beak of each *A. dauci* conidium is 200 to 250 μm x 5 μm; occasionally, the beak may have a single branch up to 100 μm in length. Conidia are seen rarely in chains of two (Simmons, 1995).

Alternaria leaf blight symptoms appear as water-soaked lesions on the foliage of the host plant, and optimum disease development occurs when the foliage remains wet for long periods (Farrar et al., 2004; Pryor and Strandberg, 2002). Spores of *A. dauci* may infect young carrot seedlings, and cause pre- and post-emergence damping-off (Bulajić et al., 2009). Even seed lots with low incidences of infection by *A. dauci* can lead to significant levels of disease in commercial carrot crops, which are typically seeded anywhere from 500,000 to 3,000,000 seeds / ha (Farrar et al., 2004). Alternaria leaf blight primarily causes damage by reducing photosynthetic activity and hindering commercial harvest when harvest is done by pulling carrot roots from the soil by the foliage. Although damping-off of carrot seedlings caused by *A. dauci*

can typically be mitigated using fungicide seed treatments like thiram and iprodione, infected or infested carrot seed can be a significant source of inoculum (Farrar et al., 2004; Maude and Bambridge, 1991). Besides infected carrot seed, mycelium on carrot debris left in the field post-harvest, and infected volunteer plants or wild carrot can be sources of *A. dauci* inoculum in commercial fields (Farrar et al., 2004).

Phylogeny of *Alternaria* diseases in carrot. *A. radicina*, *A. carotiincultae*, and *A. dauci* have been separated into two clades based on DNA fingerprinting studies with DNA sequences produced by PCR assays using protein-coding gene primers, internal transcribed spacer (ITS) ribosomal DNA (rDNA), mitochondrial small subunit (mt SSU) rDNA, and random amplified polymorphic DNA (RAPD) analysis (Hong et al., 2005; Pryor and Gilbertson, 2000; Pryor and Gilbertson, 2002). These phylogenetic studies showed that *A. radicina* and *A. carotiincultae* are related closely and belong to the radicina clade, while *A. dauci* belongs to the porri clade (Hong et al., 2005; Pryor and Gilbertson, 2000). In addition to *A. radicina* and *A. carotiincultae*, the radicina clade contains *A. petroselini* and *A. smyrni*, leaf blight pathogens of parsley, which cause little to no disease symptoms on carrot and have not been reported as carrot pathogens (Hong et al., 2005; Pryor, 2002a; Pryor and Gilbertson, 2002). Other than *A. dauci,* notable pathogens in the porri clade include *A. solani*, *A. brassicae,* and *A. porri*, which are pathogens on Solanaceae crops, Brassicaceae, and Alliaceae, respectively (Hong et al., 2005; Pryor and Gilbertson, 2002).

Hong et al. (2005) used sequences of the Alt a1 allergen gene and the glyceraldehyde-3-phosphate dehydrogenase gene (*gpd*) to separate several species of *Alternaria* into species groups. The Alt a1 allergen gene has no known function in fungal metabolism or ecology as produced by *A. alternata* (De Vouge et al., 1996). Homologs of Alt a1 are up-regulated during

infection of *Arabidopsis thaliana* plants by *A. brassisicola*, suggesting that Alt a1 may play a role in pathogenesis (Cramer and Lawrence, 2003). In the Park et al. (2008) study examining the phylogenetic relationship between *A. radicina* and *A. carotiincultae*, the Alt a1 gene significantly separated the two species into two distinct lineages, while *gpd* failed to separate the two species. Also, *A. dauci* was distinct from other species in the porri clade when using the Alt a1 gene sequences in phylogenetic studies (Hong et al., 2005; Lawrence et al., 2012). Comprehensive analysis of the Alt a1 gene consistently separates *Alternaria* spp. pathogenic on carrot into separate species, making the Alt a1 gene useful in delineating *Alternaria* spp. found to be infecting carrot seed or foliage (Hong et al., 2005; Lawrence et al., 2012; Park et al., 2008).

Management of *Alternaria* spp. in carrot crops: *Alternaria radicina* control.

Management of *A. radicina* and *A. dauci* in carrot crops is difficult and requires an integrated management approach (Farrar et al., 2004). Since the primary source of *A. radicina* inoculum can be contaminated seed, planting pathogen-free seed or treated seed is a powerful preventative measure growers can take against black rot. Carrot seed assays can be used to identify lots infected with *A. radicina* that should be treated. The International Seed Testing Association (ISTA) has approved a freeze blotter method and a malt agar method for determining the level of *A. radicina* infestation in seed lots (ISTA, 2013c; 2013d). If *A. radicina* is found on a minimum number of seed, dependent on sample size and number of replicates, in either of the seed assays, any carrot crops planted from that seed lot may be in danger of an outbreak of black rot.

If seed assays indicate that a seed lot is infected with *A. radicina*, there are options growers and seed companies can take to reduce the potential impact of seedborne inoculum on carrot crops. Seed treatments such as hot water (50°C for 20 min) and some fungicides (e.g., azoxystrobin, fludioxonil, iprodione, and thiram) can reduce seed-transmission of *A. radicina*

(Pryor, 2002b). Despite the effectiveness of most seed treatments for eradication of *A. radicina* on carrot seed, low levels of *A. radicina* may still persist after such treatments (Maude and Bambridge, 1991). Foliar fungicide applications for control of black rot in the field can be difficult after the canopy has closed because it is difficult to ensure the fungicide is applied to the carrot crown where infection is most prevalent for this pathogen. Typically, fungicide treatments are most effective when applied pre-planting or post-harvest (Farrar et al., 2004).

Crop rotation may alleviate disease pressure caused by soilborne inoculum and inoculum on post-harvest crop debris. Soil assayed from fields that had been in rotation with unrelated crops showed significantly less CFU/g soil than fields that had been in a fallow rotation (Pryor et al., 1998). In addition to examining the effects of crop rotation on *A. radicina* soil inoculum, Pryor et al. (1998) found that conidia were survival structures for *A. radicina* and could remain viable in soil for as long as four years in California. This finding was in agreement with Maude and Shuring (1972) who showed *A. radicina* was viable in soil eight years after carrot cultivation in the United Kingdom. In severely infested soils, crop rotations of greater than three to four years may be necessary for effective control of *A. radicina*.

Genetic resistance is another tool that farmers can use to control black rot. However, germplasm resistant to black rot has been developed only recently (Farrar et al., 2004). Carrot cultivars with moderate resistance to black rot are available commercially but there is still limited public information regarding resistance to black rot (Pryor et al., 2000). When colored carrot cultivars were evaluated for resistance to *A. radicina*, purple carrots showed a relatively high level of resistance to black rot compared to red, yellow, white, and orange carrots (Krämer et al., 2013). Resistance screenings have provided breeders with information on diverse genetic

backgrounds that may offer sources of resistance that can be incorporated into cultivars for growers.

***Alternaria dauci* control.** *A. dauci* can be introduced into soils through the planting of infected or infested seed and, therefore, one of the most effective preventative control measures is the use of pathogen-free or treated seed (Farrar et al., 2004; Pryor and Strandberg, 2002). The same seed assays used for detection of *A. radicina* can be used for detection of *A. dauci* in carrot seed lots but ISTA has slightly different published protocols standardized for detection of *A. radicina* and *A. dauci* in carrot seed (ISTA, 2013a; 2013b). The presence of *A. dauci* conidia on seeds may indicate that Alternaria leaf blight could develop in crops planted from the infested seed lot.

In order to reduce inoculum levels on seed, infected seed can be treated with hot water or fungicides such as azoxystrobin, fludioxonil, iprodione, and/or thiram (Strandberg, 1984). Seed treatments are generally effective for managing seedborne *A. dauci*, yet, seed treatments may not fully eradicate inoculum from carrot seed or completely prevent seed transmission which demonstrates the need for growers to utilize cultural management practices as well (Farrar et al., 2004). Crop rotation is critical for limiting infection of carrot plants by *A. dauci*. Rotations must be long enough to ensure that carrot debris decomposed, thereby eliminating a source of inoculum of *A. dauci* in fields as this fungus is not truly soilborne (Pryor et al., 2002). Carrot debris that is incorporated thoroughly into the soil will decompose quicker and, thus, *A. dauci* inoculum on such debris will be eradicated more rapidly than if debris is left on the soil surface. Carrot debris in soil that is periodically moistened will also result in less sporulation and decompose faster than debris in dry soils, indicating the importance of irrigation in drier climates vs. fallow rotations for managing this disease in carrot crops (Pryor et al., 2002).

Carrot resistance to Alternaria leaf blight is an important tool that can limit the spread and severity of *A. dauci* in growers' fields. Boedo et al. (2010) found two qualitatively different types of resistance to *A. dauci* in carrot. Resistance to Alternaria leaf blight is available in numerous publicly accessible carrot lines, and information on the general combining ability of some of these lines is available for breeders to utilize for incorporating the resistance into cultivars (Simon and Strandberg, 1998). Commercial carrot cultivars and inbred lines are available with varying degrees of resistance to Alternaria leaf blight (e.g., Bolero, Carson, B5280, 719116, 713087, and REX-240) (Pawelec et al., 2006; Rogers et al., 2002). Resistant cultivars are valuable for an integrated disease control strategy that is required for effective management of *A. dauci* in commercial carrot production.

1.4. Conclusion and research needs.

Bacterial blight in carrot can reduce photosynthetic activity and weaken the plant tops by causing foliar necrosis, and can infect the seed in carrot seed crops, making the disease a challenge to carrot root and seed growers. Although bacterial blight has had a significant impact on the carrot industry, little public research has been done to identify genetic resistance to *X. hortorum* pv. *carotae* in carrot. There is an obvious need for more effective control of bacterial blight in carrot, and the planting of resistant varieties is an important aspect to effective integrated pest management. Plant Introduction (PI) lines from the USDA-ARS National Plant Germplasm System (NPGS) have been examined and utilized in various crops, including carrot, for resistance to pests and pathogens (e.g., Condole et al., 2010; Dugan et al., 2011; Ellis et al., 1993; Mou et al., 2008; Scott et al., 1995; Villarroel-Zaballos et al., 2012). The USDA-ARS NPGS has carrot PI lines that have yet to be evaluated to identify resistance to *X. hortorum* pv. *carotae*.

Damping-off symptoms were observed on carrot seedlings during the 2012 *X. hortorum* pv. *carotae* carrot resistance screening (see Chapter 2) and, when isolations were carried out on symptomatic carrot seedlings, conidia resembling those of *A. dauci* and *A. radicina* were observed. The probable source of *A. dauci* and *A. radicina* inoculum that caused damping-off symptoms on carrot seedlings during the 2012 *X. hortorum* pv. *carotae* resistance screening was the seed of PI lines. USDA-ARS NPGS carrot PI line seed is distributed throughout the United States and world and could be a primary source of inoculum in growing areas that may not have *A. dauci* and *A. radicina* as endemic pathogens (Reitsma, 2010). The identification of pathogenic *Alternaria* spp. on PI line seed may be useful for those utilizing carrot PI line seed in commercial, public, and home research projects. Considering these research needs, the objectives of this thesis project are to:

1. Identify source(s) of genetic resistance to *X. hortorum* pv. *carotae* from a subset of *Daucus* germplasm from the USDA-ARS NPGS selected based on data from a preliminary resistance screening to foliar pathogens provided by a private seed company;
2. Correlate visual bacterial blight disease ratings of this germplasm with *X. hortorum* pv. *carotae* populations detected on the foliage to determine the efficiency of selecting for resistance to *X. hortorum* pv. *carotae* based on foliar symptoms, since the latter is far less time and resource-expensive than quantifying *X. hortorum* pv. *carotae* populations;
3. Incorporate resistance from any PI lines selected as resistant to *X. hortorum* pv. *carotae* from this screening into commercially-suitable carrot inbred line(s) to facilitate releasing the resistant lines to public and private carrot breeders; and
4. Identify *Alternaria* spp. detected on seed of *Daucus* germplasm from the USDA-ARS NPGS that was screened for resistance to *X. hortorum* pv. *carotae*.

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Chapter 2

Screening *Daucus carota* germplasm for resistance to *Xanthomonas hortorum* pv. *carotae*

2.1. Introduction

In 2012, carrot (*Daucus carota*) was cultivated for the taproot on > 29,000 ha in the United States [United States Department of Agriculture (USDA) National Agricultural Statistics Service (NASS), 2014]. Carrot growers in California led the United States with 87% of the fresh market carrot area under cultivation (USDA NASS, 2014). Washington and Wisconsin carrot growers produced the most processing carrots, with 31% of the processing carrot yield in the United States in each state in 2013 (USDA NASS, 2014). In the semi-arid, inland Pacific Northwest (Idaho, Oregon, and Washington), carrot seed growers produce 50% of the world's and 85% of the United States' carrot seed, yielding 375 kg seed/ha on an average 3,100 ha/year from 2011 to 2012 (Butler and Simmons, 2012; Mike Talkington, Nunhems USA, Inc., *personal communication*; Washington State Commission on Pesticide Registration, 2011). Washington growers produce carrot seed on approximately 1,012 ha/year with a value of $1.5 to $5.0 million [Washington State Commission on Pesticide Registration (WSCPR), 2011].

Xanthomonas hortorum pv. *carotae* causes bacterial blight of carrot (Gilbertson, 2002). Bacterial blight reduces photosynthetic activity and can hinder harvest of fresh market carrot roots when the roots are pulled from the soil by the foliage (du Toit et al., 2005; Gilbertson, 2002; Pfleger et al., 1974; Rogers et al., 2011). *X. hortorum pv. carotae* can affect carrot seed crops by blighting the umbels and foliage, reducing seed yield, and infesting developing seed (du Toit et al., 2005; Gilbertson, 2002). In the inland Pacific Northwest carrot seed production region, carrot seed crops are direct-seeded (seed-to-seed) or grown from vernalized roots (root-

to-seed) (Rubatzky et al., 1999). This carrot seed production often occurs in the same region as commercial root production, and carrot growers may encounter problems when these two crops are grown in close proximity. Volunteers, bolting (flowering) carrots left in a field from a previous season's root crop harvest, or premature bolting of carrot plants in root crops, can lead to cross pollination with carrot seed crops growing nearby [Columbia Basin Vegetable Seed Association (CBVSA), 2012]. Consecutive, direct-seeded carrot seed crop growing seasons overlap by 4 to 6 weeks, which can cause a "green bridge" effect that facilitates movement of insect pests and pathogens of carrots between nearby crops, leading to an endemic presence of some pests and pathogens (e.g., du Toit et al., 2005). *X. hortorum* pv. *carotae* can infect a newly planted seed-to-seed crop when dry, aerosolized particles, produced during threshing of a mature, infected carrot seed crop, are blown onto recently planted carrots for the next season's crops in early fall (du Toit et al., 2005). The pathogen also can be spread within a carrot seed or root crop via splashing water from precipitation or irrigation, on machinery, insects, or people moving through the plant canopy, and by plant-to-plant contact (Gilbertson, 2002).

Infested carrot seed lots can be treated with hot water or disinfectants such as trisodium phosphate or sodium hypochlorite (du Toit et al., 2005; du Toit et al., in press; Temple et al., 2013). These seed treatments typically eradicate or significantly reduce *X. hortorum* pv. *carotae* populations on seed, but are resource intensive (particularly considering the volumes of seed to be treated), and can reduce the vigor and shelf-life of carrot seed lots. Therefore, production of carrot seed that is not infested with this pathogen is preferable to having to treat carrot seed.

Chemical management options for bacterial blight are limited for various reasons. Symptoms are not expressed in crops until *X. hortorum* pv. *carotae* populations reach 10^5 to10^6 CFU/g dry foliage (du Toit et al., 2005; Gilbertson, 2002). Consequently, chemical applications

made after the appearance of bacterial blight symptoms largely are ineffective because the most commonly used bactericides are copper products that are entirely protective, not curative. du Toit and Derie (2005, 2008b) and du Toit et al. (2006) demonstrated that copper bactericides (e.g., copper hydroxide) can reduce significantly the development of *X. hortorum* pv. *carotae* populations on carrot foliage, but effective control of the pathogen could not be achieved after the pathogen had established on carrot foliage. Protective applications of copper hydroxide, with or without mancozeb (Kocide and ManKocide, respectively; DuPont, Wilmington, DE), significantly limited development of the population of *X. hortorum* pv. *carotae* on carrot foliage only if the applications were made prior to inoculation of plants with the pathogen, compared to non-treated, inoculated plants (du Toit and Derie, 2008a).

Cultural practices can help mitigate the spread of *X. hortorum* pv. *carotae* between and within carrot root and seed crops. Since the duration of carrot leaf wetness and splashing water are important in development and spread of *X. hortorum* pv. *carotae*, drip and furrow irrigation can be effective for managing bacterial blight (du Toit et al., 2005; Gilbertson, 2002; Gugino et al., 2004; Weber et al., 2004). Approximately 75% of carrot seed growers in Oregon have switched from overhead to drip irrigation in the past 10 years to optimize water usage, increase seed yields, and manage bacterial blight (Weber et al., 2004). Crowe et al. (2005) showed that carrot seed lots harvested from drip-irrigated crops typically have reduced *X. hortorum* pv. *carotae* populations and increased germination compared to seed harvested from sprinkler irrigated carrot seed crops. However, drip irrigation alone may not eliminate or protect carrot crops entirely from infection by *X. hortorum* pv. *carotae,* and harvested seed lots can still be infested with *X. hortorum* pv. *carotae* (Crowe et al., 2005; du Toit et al., 2005). Incorporating carrot root or seed crop debris into the soil after harvest to facilitate residue decomposition, and

practicing a two- to three-year crop rotation may help minimize infection of future carrot crops from *X. hortorum* pv. *carotae*-infested carrot debris (Gilbertson, 2002; Gugino et al., 2004).

When evaluating commercial carrot cultivars, a carrot breeding line, and a carrot Plant Introduction (PI) line for resistance to bacterial blight, Pfleger et al. (1974) showed that the degree of susceptibility to bacterial blight varied among these genotypes. However, there has been little emphasis publicly on the breeding of carrot for resistance to bacterial blight, and no carrot cultivars currently are marketed as resistant to bacterial blight (Boiteux and Simon, 2002; Gugino et al., 2004). Commercial grower demand for *X. hortorum* pv. *carotae*-free seed has superseded the necessity for bacterial blight-resistant varieties in carrot, and recent development of threshold based approaches to *X. hortorum* pv. *carotae* management in root crops have necessitated a greater understanding of carrot genotype influence on disease development (i.e., highly susceptible varieties are more risky) (Peter Rogers, Nunhems USA, Inc., *personal communication*). Nevertheless, there have been recent efforts to breed for resistance to *X. hortorum* pv. *carotae* in the private seed industry but no cultivars marketed as resistant to bacterial blight have yet been released (Peter Rogers, *personal communication)*.

Kimbrell et al. (2011) showed that orthologs of type III effector genes responsible for resistance-gene mediated responses in tomato and pepper are present in the genome of *X. hortorum* pv. *carotae*. Additionally, Leite et al. (1994) showed that homologs of *hrp* genes of *X. campestris* pv. *vesicatoria* are also present in *X. hortorum* pv. *carotae*. Type III effector genes trigger hypersensitive resistance responses in tomato and pepper to *X. campestris* pv. *vesicatoria,* and *hrp* genes, which are associated with symptoms produced on susceptible host plants and hypersensitive reactions in resistant host plants inoculated with certain pathogens (Minsavage et al., 1990; Schulte and Bonas, 1992; Stall et al., 2009; Willis et al., 1991). The presence of these

genes in *X. hortorum* pv. *carotae* (Kimbrell et al., 2011) indicates there may be more effective levels of resistance to bacterial blight within carrot germplasm than the resistance currently available in commercial cultivars. However, there is relatively limited genetic diversity in cultivated, orange-rooted carrots (Pierre and Bayer, 1991). This genetic bottleneck may limit the ability of carrot breeders to select novel traits, such as disease resistance, from orange-rooted carrots.

X. hortorum pv. *carotae* has had a significant impact on the carrot industry, yet there has been little public research to identify genetic resistance to this pathogen. Accessions in plant germplasm collections have been evaluated in various vegetable crops for novel traits such as disease resistance (e.g., Condole et al., 2010; Dugan et al., 2011; Ellis et al., 1993; Mou et al., 2008; Scott et al., 1995; Villarroel-Zaballos et al., 2012). The USDA National Plant Germplasm System (NPGS) has 1,370 accessions of *Daucus* spp. from 64 countries (Reitsma and Clarck, 2013). Carrot accessions from the USDA NPGS are diverse, spanning 31 species and subspecies (Reitsma and Clarck, 2013). The accessions have been used to identify resistance to carrot fly, which has been incorporated into commercially-acceptable carrot germplasm (Ellis, 1999). The USDA NPGS could offer a source of novel resistance to other carrot pests and pathogens, including *X. hortorum* pv. *carotae*. Therefore, the objectives of this research were to: 1) identify source(s) of genetic resistance to *X. hortorum* pv. *carotae* from a subset of *Daucus* germplasm from the USDA-Agricultural Research Service NPGS, with the subset selected based on data from a preliminary resistance screening to foliar pathogens provided by a private seed company (*data not shown*); 2) correlate visual bacterial blight disease ratings of this germplasm with *X. hortorum* pv. *carotae* populations detected on the foliage to determine the efficiency of selecting for resistance to *X. hortorum* pv. *carotae* based on foliar symptoms, since the latter is far less

time and resource-expensive than quantifying *X. hortorum* pv. *carotae* populations; and 3) incorporate resistance from any PI lines identified as resistant to *X. hortorum* pv. *carotae* from this screening into commercially-suitable carrot inbred line(s) to facilitate making the resistance available to public and private carrot breeders.

2.2. Materials and Methods

2012 resistance screening. ***Planting and trial maintenance.*** Two-hundred seeds of each of 66 carrot PI lines were obtained from the North Central Regional Plant Introduction Station (NCRPIS) USDA NPGS (Table 2.1). These PIs were selected based on preliminary screening data provided by a carrot breeder for a commercial seed company. The PIs that appeared most resistant or most susceptible to bacterial blight, Alternaria leaf blight, and/or Cercospora leaf spot were selected from the preliminary screening. Two inbred male-sterile carrot lines (A2566 and A0493) were obtained from Dr. Phil Simon's USDA-ARS carrot breeding program at the University of Wisconsin for making crosses with selected PI lines based on reactions of the PIs to inoculation with *X. hortorum* pv. *carotae*, to introgress the selected resistance into a stable genetic background with horticultural traits acceptable for commercial carrot cultivation. Commercial carrot cultivars from Bejo Seeds, Inc.; Nunhems USA, Inc.; Sakata Seed America; and Monsanto Vegetable Seeds, Inc. were obtained from company representatives, breeders, and Osborne International Seed Co. to compare results for the PIs to industry standard cultivars. Seeds of the proprietary cultivars were requested based on commercial popularity of the cultivars, and to represent a diversity of carrot types.

In September 2012, carrot seeds of each PI line, male-sterile inbred line, and cultivar were planted in RediEarth Starter Medium (SunGro Horticulture, Vancouver, BC, Canada) in

PL-72 standard plug trays (T.O. Plastics, Otsego, MN). The seed of each PI line were not hot water treated for *X. hortorum* pv. *carotae* infection because of concerns that hot water treatment might reduce vigor of some of the PI seed lots. Each flat was filled with potting mix and imbibed in fertigated (Wil-Sol Pro-Balance 20-20-20 Superior Water Soluble Professional Turf and Ornamental Fertilizer, Wilbur-Ellis, San Francisco, CA) water injected at 200 ppm N. Seeds were planted (1 seed/cell) at a depth of 0.5 cm, and the flats watered after planting. Carrot plants were grown with a 10-h photoperiod, a maximum day temperature of 26°C, and a maximum night temperature of 25°C. Flats were fertigated as needed. Insect pests were controlled with a weekly spray of acephate (Orthene Turf, Tree, and Ornamental Spray 97, Amvac Chemical Corp., Los Angeles, CA), spinosad (Entrust Naturalyte Insect Control, Dow AgroScience LLC, Indianapolis, IN), imidacloprid (Leverage 2.7, Bayer CropScience LP, Research Triangle Park, NC), or *Beauvaria bassiana* (Botanigard 22WP, Laverlam International Corp., Butte, MT), with the products rotated weekly. When spider mites (*Tetranychus* spp.) were detected on the foliage, abamectin (Avid 0.15EC, Syngenta Crop Protection, LLC, Greensboro, NC) was sprayed on carrot foliage.

At the three- to four-leaf growth stage, approximately one month after planting, seedlings were transplanted into Sunshine Mix #1 potting medium (SunGro Horticulture) in D40 deepots (Stuewe and Sons, Inc., Tangent, OR). Each deepot was filled to within 2.5 cm of the top of the pot to facilitate deep watering, since the long slender shape of the deepots necessitated watering deeply to promote deep rooting of the carrots. Twenty-five vigorous plants of each line were selected from the 72 cell tray, the root plug of each was dislodged from the tray, and the seedling placed in a deepot filled with potting mix. Deepots were placed in a 35.6 cm by 35.6 cm N25T rack (Stuewe and Sons, Inc.), with up to 25 deepots/rack. The ribs on the side of each deepot

were removed using a bench grinder so that the deepot slid into the N25T tray. Racks containing individual PI lines were randomized on the greenhouse bench. However, each plant (replicate) of a PI line was not randomized within racks because of greenhouse space constraints. Imidacloprid (Marathon 1% G, OHP, Inc, Mainland, PA) was applied to the top of the potting soil around the carrot plant in each deepot immediately after transplanting, for insect control. Ten weeks after planting and one day prior to inoculation, each rack of 25 deepots was enclosed in a large, 0.05 mm thick, 81.28 cm x 71.12 cm x 152.40 cm plastic bag (U.S. Plastic Corp., Lima, OH), and the bag closed to increase relative humidity and promote opening of stomata on the carrot foliage to facilitate infection by *X. hortorum* pv. *carotae*.

Inoculation. Five days prior to inoculating the carrot plants, isolate Car111 of *X. hortorum* pv. *carotae*, obtained from carrot seed in 2003 by the Vegetable Seed Pathology program at the Washington State University (WSU) Mount Vernon Northwestern Washington Research and Extension Center (NWREC), and demonstrated previously to be pathogenic on carrot (du Toit et al., 2005), was taken out of long-term storage at -80°C and grown on yeast dextrose calcium carbonate (YDC) agar medium (Schaad, 1988) at approximately 20°C. After 4 days, one bacterial colony was selected from the YDC agar plates and, with an inoculation loop, placed in 50 mL of 523 broth (Kado and Heskett, 1970) in a 125 mL Erlenmeyer flask. Inoculated flasks (n = 15) of 523 broth were placed on a shaker at 200 rpm at 28°C for 16 h, after which the 523 broth was pooled from all flasks into a 500 ml flask.

The bacterial concentration used to inoculate plants in the 2012 screening was 10^8 CFU/ml. A DU-65 spectrophotometer (Beckman Coulter, Inc., Brea, CA) was used to adjust the bacterial suspension to 0.10 optical density (OD) at 540 nm (du Toit et al., in press). For calibration, 1 ml of non-inoculated 523 broth was placed in a cuvette in the spectrophotometer.

A 25 µl aliquot of the bacterial suspension was then placed in a cuvette with the non-inoculated 523 broth, and OD measured. Five microliter aliquots of the bacterial suspension were added to the cuvette until an OD of 0.10 was reached, and the appropriate dilution carried out to obtain 10^8 CFU/ml in 5 liters of 12.5 mM PO_4 buffer. A 100 µl aliquot of the bacterial suspension was spread onto each of three plates of 523 agar medium in a dilution series to confirm the inoculum concentration applied to the carrot foliage (1.5×10^8 to 1.8×10^8 CFU/ml).

A pressurized Model T sprayer (Bellspray, Inc., Opelousas, LA) was used to inoculate the carrot foliage. Individual racks of 25 carrots were each taken off the greenhouse bench and placed on the ground. The plastic bag covering the rack was removed, and the plants sprayed until the foliage was visibly wet (approximately 58 ml/rack). The plants were enclosed again in the plastic bag, and placed back on the bench. Approximately 72 h after inoculation, the bag was removed from each rack and the rack placed in a 40 cm x 40 cm x 12.7 cm bin (Anderson Die and Manufacturing Inc., Portland, OR) lined with 0.05 mm-thick Husky plastic sheeting (Poly-America, Grand Prairie, TX) so that the plants could be watered by imbibition from the bottom of each deepot. Plants were not watered from above after inoculation because *X. hortorum* pv. *carotae* is spread readily by splashing water. A bamboo stake (70.5 cm long) was placed in a deepot in each of the four corners of each rack of 25 deepots, and string tied around the four stakes to lift the foliage and minimize contact between adjacent lines. This tying up of the foliage was repeated periodically as the foliage grew. Throughout the resistance screening trial, 70% isopropanol was used to sanitize hands and tools after handling foliage of each line to minimize cross-contamination among lines.

Sampling and disease assessment. After inoculation, plants were monitored weekly for appearance of symptoms of bacterial blight, and notes were taken based on when each line first

expressed symptoms. Visual ratings of bacterial blight symptoms were done for each line 4 and 5 weeks post-inoculation (wpi) based on the percentage foliage blighted for all 25 plants in a rack, on a 0-to-10 scale, where: 0 = 0% of the foliage blighted, and 1 to 10 represent 10% increments of the foliage with bacterial blight symptoms, up to 100% foliage blighted. The average percentage blighted foliage was estimated visually for each rack by the same person to avoid bias among raters.

Approximately 6 wpi, individual plants were sampled and tested for the amount of *X. hortorum* pv. *carotae* on the foliage using a dilution plating method (du Toit et al., 2005). Bacterial blight severity of the foliage of each plant was rated the day each plant was processed for dilution plating. Foliage was sampled diagonally across each rack from five plants in order to capture variation in canopy microclimate across the rack. Foliage of each plant was cut 1.5 to 2.0 cm above the crown, and rated for percentage bacterial blight symptoms as previously described. Foliage from each plant was then weighed and placed in a paper bag marked with the line and sample number. Scissors, pruning shears, and the hands of people sampling were sterilized with 70% isopropanol between samples. On the same day, the foliage was cut into pieces approximately 5 to 10 mm^2, and placed in phosphate buffer (12.5 mM PO_4). If the total foliage of a plant weighed 40 to 55 g, the cut foliage was placed into 250 ml buffer; if 25 to 39 g, the foliage was placed in 200 ml buffer; 10 to 24 g foliage was placed in 150 ml buffer; 5 to 9 g foliage in 100 ml buffer; and <5 g foliage in 50 ml buffer. The foliage and buffer were placed on a gyratory shaker at 250 rpm for 1 h.

A 10-fold dilution series was then prepared by pipetting 1 ml of the foliar wash into 9 ml sterilized phosphate buffer in a test tube, and subsequent dilutions done similarly. Depending on severity of the visual foliar rating for each plant, the dilution was carried out to 10^{-5} to 10^{-6}, with

greater dilutions for plants with more severe foliar blighting. A 100 µl aliquot of each dilution was pipetted onto a plate of XCS semi-selective agar medium (Williford and Schaad, 1984), and spread over the surface of the plate with a sterilized, bent glass rod. Three replicate plates were used for each dilution. For 10^{-2} to 10^{-7} dilutions, three 10 µl aliquots of each dilution were spread across one plate with adequate space between adjacent streaks to avoid mixing the aliquots. The plates were placed in the dark in incubators (Model I-30BLL and Model I-30VL, Percival Scientific, Perry, IA) set at 28°C for 3 to 5 days. The foliage in the buffer solution in each flask was then separated in a strainer, rolled in a paper towel, and placed in a paper bag. The paper bag and foliage were then dried in an oven for 3 to 7 days, and foliar dry weight recorded. After 3 days of incubation, the XCS agar plates for those dilutions that produced visibly distinct colonies were used to quantify colonies typical of *X. hortorum* pv. *carotae*. For the three streak plates/dilution, each streak was counted as an individual subsample. Yellow, mucoid, raised colonies typical of this pathogen (du Toit et al., in press) were enumerated for each replicate of each dilution streak.

Root vernalization and planting. When the resistance screening was completed and data analyzed (see the Results section), carrot lines exhibiting the greatest putative resistance and those with the most susceptible reactions to *X. hortorum* pv. *carotae* were placed in cold storage to vernalize the roots. Putative resistant and susceptible lines were selected based on the amount of *X. hortorum* pv. *carotae* recovered from carrot foliage, not on visual disease ratings, because reactions of the PI lines were distinguished more definitively based on bacterial populations detected on the foliage than foliar disease ratings (see Results section). Selection of the eight putative resistant PI lines from the 2012 screening was carried out using the mean $\log_{10}$ CFU/g dry foliage for each line. This included PIs with a mean of < 8.000 $\log_{10}$ CFU/g dry foliage and

two PI lines that had at least two plants with < 8.000 $\log_{10}$ CFU/g dry foliage. In addition, four cultivars with the greatest bacterial populations, and four cultivars with the least average bacterial populations detected on the foliage were selected. PI lines 163238, 176969, 177381, 263601, 418967, 432905, 432906, and 436674 were selected as resistant to *X. hortorum* pv. *carotae,* and PIs 226636, 234621, 277710, 390887, and 390893 were selected as susceptible to the pathogen, for subsequent vernalization. Additionally, plants of the two male-sterile inbred carrot lines (A0493 and A2566) were vernalized. The selected roots were removed from the deepots and submersed in a 53 mg/liter suspension of boscalid + pyraclostrobin (Pristine, BASF Corp., Florham Park, NJ) for 15 sec to minimize storage rot from *Alternaria radicina*, which had been detected on seedlings and roots of some PIs during this trial (see Chapter 3). Roots of each selected line were then drip-dried and placed in a paper bag, for all 25 roots of each selected PI line, and up to 10 roots of each of the two inbred lines. A volume of white pine shavings (GEM Shavings LLC, Auburn, WA) equivalent to the volume of carrot roots was placed in each bag. Each paper bag was then placed in a gusseted, 30.5 cm x 20.3 cm x 76.2 cm plastic bag (U.S. Plastic Corp.), and the bag closed. Holes were punched in each bag to facilitate gas exchange. The bags were then placed in the dark at 4 ± 1°C for 10 weeks.

The carrot roots were removed from the bags in cold storage in March 2013, after 10 weeks of vernalization, and planted in a greenhouse. Any rotted and/or desiccated roots were discarded. Each root was planted into Sunshine Mix #1 potting medium in a 2.8 liter pot (Polycan No. 1 Deep, Anderson Die and Manufacturing, Inc.). Pots were placed in the greenhouse under the growing conditions described above. However, the roots of all of the selected PI lines either desiccated or succumbed to storage rot when removed from cold storage

after 10 weeks, which prevented making crosses of the selected lines from the 2012 screening with the male-sterile inbreds.

2013 resistance screening. ***Planting and trial maintenance.*** Seed of each of 15 carrot wild relatives were obtained from Dr. Phil Simon with the USDA-ARS at the University of Wisconsin in Madison, WI (Table 2.3). Seed also was collected from a wild carrot growing in Bay View, WA by Charles E. Christianson, and seed of four cultivars (one cultivar selected for resistance to *X. hortorum* pv. *carotae*, and three cultivars susceptible to *X. hortorum* pv. *carotae*) were obtained from Nunhems USA, Inc. Seed of these 20 additional carrot lines, the 13 PI lines selected from the 2012 trial, an additional 2 PI lines that had abnormalities in foliar growth in the 2012 trial, 8 commercial (open pollinated and hybrid) cultivars from the 2012 trial, and the 2 inbred male-sterile lines evaluated in 2012 were planted into a 2.5 cm layer of RediEarth Starter Medium on top of Sunshine Mix #1 potting mix in D40H deepots. Two seeds were planted/pot, and the seedlings thinned to one plant/pot two weeks after planting. After four weeks, the deepots were randomized into five complete blocks with five subsamples (plants)/block. Two PIs or cultivars lines were placed in each rack, with five plants of one line on one side of the rack and five plants of another line on the opposite side of the rack. Similar to the 2012 trial, irrigation bins were set up to water the plants from below, and plant foliage was tied up prior to inoculation and at regular intervals to avoid contact of the foliage between adjacent lines.

Nutritional deficiency symptoms were observed on carrot foliage four weeks after planting, so a potting medium sample was sent to Soiltest Farm Consultants, Inc. (Moses Lake, WA) for analysis. The potting medium was deficient in boron (B) and copper (Cu). Therefore, a foliar application of B (NUE Boron 4%, BioGro, Inc., Mabton, WA) was made 6 weeks after planting, and daily fertigated applications of Cu and sulfur (S, Cop-Plex, Northwest Agricultural

Products, LLC, Pasco, WA) were used to compensate for the B and Cu deficiencies in the medium.

Inoculation. The same inoculation protocol from the 2012 trial was followed for the 2013 trial, except that a total of 89.3 ml of a *X. hortorum* pv. *carotae* suspension of 10^8 CFU/ml was applied to the 25 plants (5 replicates of 5 plants) of each line, and a 0.03 mm thick, 96 cm x 130 cm plastic bag (HDX, Home Depot, Atlanta, GA) was used to enclose the plants in each rack.

Sampling and disease assessment. The percentage carrot foliage blighted was recorded 2, 3, and 4 wpi for each set of five plants/line/replicate block. At 6 wpi, plants were sampled by cutting the foliage of each plant as described for the 2012 trial. Some plants had senesced before sampling, resulting in < 25 plants sampled for those lines. The cut foliage was placed in a cooler overnight at 4°C. The same method was used for preparing and dilution plating the foliar wash as in the 2012 trial, except that for plants with 26 to 55 g foliage, the cut foliage was placed into 250 ml buffer; for plants with 11 to 25 g foliage, the foliage was placed in 150 ml buffer; and for plants with ≤ 10 g foliage, the foliage was placed in 100 ml buffer. Bacterial colony counts and foliar dry weights were recorded as in the 2012 screening.

Root vernalization and planting. All lines that had a mean *X. hortorum* pv. *carotae* population detected 6 wpi that was statistically similar to that of PI 418967 were selected as putatively resistant to the pathogen, since PI 418967 had the smallest mean *X. hortorum* pv. *carotae* population recovered of all the lines and cultivars tested (see Results section). Susceptible selections included all lines that had *X. hortorum* pv. *carotae* recovered populations similar statistically to that of Ames 26381, since this line had the greatest mean pathogen population detected. In addition, PI 357984 was selected as susceptible to *X. hortorum* pv.

carotae for making crosses to inbred A2566 and A0493 because roots of this PI survived vernalization whereas few other susceptible PIs survived vernalization. Roots of selected putative resistant and susceptible lines were kept in the potting medium in the original deepots, and placed in cold storage for vernalization. Healthy roots of PIs 357984, 390893, 418967, 432905, 432906, Ames 26381, and Ames 26384, and the male-sterile inbred lines A2566 and A0493, were placed in the dark at 4 ± 1°C for 10 weeks. Each root was then planted into Sunshine Mix #1 potting medium in a 2.8 liter pot (Polycan No. 1 Deep) in a greenhouse with a 17-h daylength.

In addition to planting these vernalized roots from the 2013 screening, seed of each of PIs 390893, 418967, 432905, 432906, Ames 26381, and Ames 26384 were grown for 6 weeks in 72-cell flats in Sunshine Mix #1 in September to October 2013, and the flats then placed at 4 ± 1°C for 10 weeks to vernalize the seedlings. Half the flat of each PI was kept in the dark while the other half of each flat was kept under 12 h/12 h day/night cycle (each flat was cut in half). The additional plants of these PIs were planted because most or all roots of these PIs had rotted or senesced in cold storage or shortly after transplanting. The vernalized seedlings were removed from cold storage and two plants/PI planted in Sunshine Mix #1 potting medium as described above.

***Crosses and pollination*.** Vernalized carrot plants were monitored for initiation of flowering. When a plant began to produce umbels, the king umbel was placed in a pollination cage along with an umbel of inbred A2566 and/or A0493. Pollination cages were made by creating a tube from 1 cm x 1 cm wire mesh that was approximately 23 cm diameter and 36 cm tall. A thin metal wire was attached to each side of one end of the mesh tube, and the wire placed through an approximately 13.0 cm long section of 1.9 cm-diameter PVC pipe. A standard

size (51 cm x 66 cm) pillow case was cut to form a cloth tube that was placed over the wire frame. One end of the pillow case was tied around the PVC pipe, and the cage placed over the umbels to be pollinated. The other end of the pillow case was then tied around the stems of the carrot plants so that the only opening to the cage was through the PVC pipe. Blue bottle fly pupae [Calliphoridae (Genus and species not specified), Forked Tree Ranch, Bonners Ferry, ID] were introduced into each cage through the opening in the PVC pipe, and the opening was then sealed with a cork or paper towels. A majority of the blue bottle flies appeared lethargic and had deformed wings. Therefore, house flies (*Musca domestica*, Rincon-Vitova, Ventura, CA) were used for subsequent pollinations, as the latter were more active than the blue bottle flies.

Data analysis. The 2012 and 2013 data were analyzed using SAS Version 9.2 (SAS Institute, Cary, NC). The population of *X. hortorum* pv. *carotae* recovered/plant (CFU/g dry foliage) was calculated based on CFU/ml buffer and the ratio of total buffer volume to foliar dry weight that resulted in quantifiable bacterial populations. Data for CFU/g dry foliage were log-transformed [$y = \log_{10}$(CFU/g dry foliage)]. The data for $\log_{10}$ CFU/g dry foliage for all lines and cultivars tested in 2012 and 2013 did not meet the assumptions of normally distributed residuals and homogeneity of variance required for parametric analysis. Additionally, arcsin and square root-transformations of the dilution plating data did not satisfy the assumptions of normally distributed residuals and homogeneity of variance required for parametric analysis. Therefore, Friedman's non-parametric rank test and Fisher's protected least significant difference (LSD at $P < 0.05$) were carried out on ranked pathogen population data ($\log_{10}$ CFU/g dry foliage). Normality and homogeneity of variance assumptions, and means comparisons using the least squares means of the fixed effect of carrot lines were calculated for the ranked data using PROC UNIVARIATE, PROC GLM, and PROC MIXED, respectively. Spearman's

correlation coefficients were calculated for the 2012 disease severity ratings 4, 5, and 6 wpi, as well as $\log_{10}$ CFU/g dry foliage detected 6 wpi. Similarly, Spearman's correlation coefficients were calculated for the 2013 resistance screening data for foliar ratings 2, 3, 4, and 6 wpi, and for $\log_{10}$ CFU/g dry foliage detected 6 wpi.

2.3. Results

2012 resistance screening. Bacterial blight symptoms were observed on at least one, and typically all, inoculated plants of the male-sterile inbred lines, PI lines, and cultivars evaluated in the 2012 greenhouse screening, demonstrating the success of the inoculation protocol for establishing the disease. No symptoms were observed on plants of the non-inoculated inbred lines when evaluated 4 and 5 wpi. All but one non-inoculated male-sterile inbred plant had 0% foliage blighted 6 wpi; the one plant that developed symptoms had 3% foliage blighted compared to an average 8.8% blighting for all inoculated plants. For most of the entries, symptoms began as chlorotic lesions on the foliage that developed into angular, water-soaked lesions and eventually brown to black, necrotic lesions (Fig. 2.1A, 2.1B, and 2.1C). However, there were variations in symptoms among entries (Fig. 2.1D and 2.1E). Symptoms on PI 288458 started as purple discoloration along the margins of the foliage, which progressed into necrotic lesions (Fig. 2.1D). PI 226636 produced similar purple symptoms; however, the necrotic lesions were lighter colored and more pronounced than on PI 288458 (Fig. 2.1E). Disease severity varied extensively among the PIs and cultivars at 4, 5, and 6 wpi (Table 2.1 and Fig. 2.2A).

Severity of foliar blighting ranged from 0 to 30% for individual entries at both 4 and 5 wpi, with a mean foliar rating of 8.0 ± 0.7 and 9.1 ± 0.7% for all 85 lines and cultivars tested, respectively (Table 2.1). By 6 wpi, disease ratings on individual plants ranged from 0 to 50%

and averaged 8.8 ± 0.4% for all lines tested (Table 2.1). The 66 PI lines averaged 8.5 ± 0.9 and 9.5 ± 0.9% severity, and ranged from 0 to 30% foliar blighting 4 and 5 wpi, respectively; whereas cultivars averaged 7.1 ± 1.4 and 8.2 ± 1.2% severity, and ranged from 0 to 20 and 5 to 20% foliar blight severity 4 and 5 wpi, respectively. By 6 wpi, the PIs averaged 9.3 ± 0.5% foliage blighted, and ranged from 0 to 50% severity vs. 8.8% severity with a range of 0 to 30% foliar blighting for the 17 commercial cultivars evaluated. Male-sterile inbred lines averaged 6.3 ± 0.8, 6.3 ± 0.8, and 5.4 ± 0.6% foliar blighting; and ranged from 5 to 10, 5 to 10, and 0 to 20% severity 4, 5, and 6 wpi, respectively.

Spearman's correlation coefficients for disease ratings 4 vs. 5 vs. 6 wpi were highly significant for all 85 lines and cultivars tested (r = 0.4432 to 0.8868 at $P < 0.0001$ for all pairs of ratings) (Table 2.2). Similarly, Spearman's correlation coefficients for disease ratings 4 vs. 5 vs. 6 wpi were highly significant for all 66 PIs tested (r = 0.4236 to 0.8658 at $P < 0.0001$ for all pairs of ratings) (Table 2.2). Likewise, for the 17 cultivars tested, with r = 0.8105 ($P < 0.0001$), 0.5902 ($P = 0.0126$), and 0.6873 ($P = 0.0023$) for disease ratings at 4 vs. 5, 4 vs. 6, and 5 vs. 6 wpi, respectively (Table 2.2). For the 2012 resistance screening data, Friedman's rank test of $\log_{10}$ CFU/g dry foliage and disease severity ratings 6 wpi showed a significant effect of carrot line/cultivar on the population of *X. hortorum* pv. *carotae* recovered from the foliage ($P < 0.0001$) as well as severity of symptoms observed on the foliage 6 wpi ($P < 0.0001$).

X. hortorum pv. *carotae* was recovered from every inoculated plant among the 66 PI lines, 17 cultivars, and 2 male-sterile inbred lines tested. Bacterial populations on foliage of all 85 lines and cultivars ranged from $\log_{10}$ 4.140 to $\log_{10}$ 11.516 CFU/g dry foliage with a mean ± SE of $\log_{10}$ 9.230 ± 0.048 CFU/g dry foliage (Table 2.1). The pathogen was not detected on 8 of 15 non-inoculated male-sterile inbred plants. In addition, three of the non-inoculated control

plants had ≤ $\log_{10}$ 4.000 CFU/g dry foliage, and the four remaining control plants had populations ranging from $\log_{10}$ 5.084 to $\log_{10}$ 7.799 CFU/g dry foliage. Bacterial blight symptoms did not appear until *X. hortorum* pv. *carotae* populations were > $\log_{10}$ 6.000 CFU/g dry foliage and, as symptoms became more severe, bacterial populations increased at a diminishing rate (Fig. 2.2A). However, Spearman's correlation coefficients for *X. hortorum* pv. *carotae* populations vs. foliar disease ratings 4, 5, and 6 wpi were highly significant ($P < 0.0001$) for all 85 lines tested (r = 0.5416, 0.5036, and 0.5183, respectively) (Table 2.2). The interquartile range (middle 50% of data) of all plants tested had *X. hortorum* pv. *carotae* populations of $\log_{10}$ 8.827 to $\log_{10}$ 9.870 CFU/g dry foliage (Fig. 2.3A), as a gauge of potential genetic variability within groups of lines and cultivars. Cultivars, PI lines, and male-sterile inbred lines averaged $\log_{10}$ 9.335 ± 0.101, $\log_{10}$ 9.218 ± 0.059, and $\log_{10}$ 9.105 ± 0.121 CFU/g dry foliage with a range from $\log_{10}$ 5.420 to $\log_{10}$ 11.516, $\log_{10}$ 4.140 to $\log_{10}$ 11.370, and $\log_{10}$ 6.803 to $\log_{10}$ 10.949 CFU/g dry foliage, respectively. The interquartile range in pathogen population detected on the cultivars, PI lines, and male-sterile inbred carrot lines A2566 and A0493 overlapped considerably, and ranged from $\log_{10}$ 8.860 to $\log_{10}$ 9.866, $\log_{10}$ 8.827 to $\log_{10}$ 9.906, $\log_{10}$ 8.884 to $\log_{10}$ 9.736, and $\log_{10}$ 8.440 to $\log_{10}$ 9.425 CFU/g dry foliage, respectively (Fig. 2.3A).

X. hortorum pv. *carotae* populations vs. disease ratings 4, 5, and 6 wpi for the 66 PI lines were all highly significantly correlated ($P < 0.0001$), with r = 0.6188, 0.6004, and 0.5295, respectively (Table 2.2). In contrast, Spearman's correlation coefficients of *X. hortorum* pv. *carotae* population vs. disease rating 4 and 5 wpi for the 17 cultivars evaluated were not significant, whereas *X. hortorum* pv. *carotae* population vs. disease rating 6 wpi was correlated highly significantly, with r = 0.5635 ($P < 0.0001$) (Table 2.2). Spearman's correlation coefficients of pathogen population vs. foliar disease ratings for the two inbred male-sterile lines

were not correlated significantly (*data not shown*). Several PIs appeared as outliers for the entire set of entries evaluated because of limited mean bacterial populations detected on the foliage despite relatively severe foliar disease ratings, i.e., PIs 163238, 418967, 432900, and 436674 which had mean foliar ratings of 8.0, 7.4, 15.4, and 8.0% foliage blighted, respectively, vs. mean bacterial population recovered of $\log_{10}$ 7.866, $\log_{10}$ 6.398, $\log_{10}$ 8.085, and $\log_{10}$ 7.479 CFU/g dry foliage, respectively (Table 2.1 and Fig. 2.2A).

No foliar symptoms were observed on PI 176969 at both 4 and 5 wpi. This PI had the least severe foliar blight 6 wpi with a mean of 2.0 ± 1.4% foliage blighted (range of 0 to 7%), which was similar statistically to that of 28 other PI lines and cultivars (Table 2.1). PI 176969 ranked the third least in bacterial population recovered from the foliage with $\log_{10}$ 7.319 ± 0.621 CFU/g dry foliage (range of $\log_{10}$ 5.280 to $\log_{10}$ 8.651 CFU/g dry foliage), which was similar statistically to that of 20 PIs and cultivars (Table 2.1). PI 418967 had the least *X. hortorum* pv. *carotae* recovered from foliage among all the lines and cultivars tested ($\log_{10}$ 6.398 ± 0.498 CFU/g dry foliage, with a range of $\log_{10}$ 4.549 to 7.485 CFU/g for individual plants), but this population was similar statistically to that of 18 other PIs and cultivars (Table 2.1). Foliar disease ratings 4 and 5 wpi for this PI were 0 and 5%, respectively (Table 2.1). However, PI 418967 ranked 47 out of 85 lines and cultivars screened for foliar disease severity 6 wpi, with a mean of 7.4 ± 1.1% foliage blighted (range of 5 to 10% for individual plants), which was statistically similar to that of 75 other PIs and cultivars (Table 2.1).

In contrast to PI 176969 and 418967, PI 284773 had 10 and 20% foliar severity ratings 4 and 5 wpi, and the greatest average disease severity 6 wpi (27.4 ± 9.5% foliage blighted, with a range of 7 to 50% for individual plants). The mean foliar rating of this PI 6 wpi was similar statistically to that of 35 other PIs and cultivars (Table 2.1). Additionally, PI 284773 ranked 71

out of 85 for mean population of *X. hortorum* pv. *carotae* detected on foliage 6 wpi with $\log_{10}$ 9.872 ± 0.060 CFU/g dry foliage ($\log_{10}$ 9.690 to $\log_{10}$ 10.000 CFU/g for individual plants) which was similar statistically to that of 54 PIs and cultivars (Table 2.1). PI 226636 had the greatest population of *X. hortorum* pv. *carotae* recovered from foliage among all the lines and cultivars tested, with $\log_{10}$ 10.465 ± 0.152 CFU/g dry foliage ($\log_{10}$ 10.004 to $\log_{10}$ 10.871 CFU/g), which was similar statistically to that of 30 other PIs and cultivars (Table 2.1). This PI had 20% foliage blighted at both the 4 and 5 wpi ratings, and 12.4 ± 3.2% foliage blighted 6 wpi. The latter rating ranked 82 out of 85 lines and cultivars (range of 5 to 20%), and was similar statistically to the mean foliar disease ranking of 34 other PIs or cultivars (Table 2.1).

In addition to PIs 176969 and 418967, PIs 163238, 177381, 263601, 432905, 432906, and 436674 were selected as putative resistant lines to *X. hortorum* pv. *carotae* for backcrossing to the two male-sterile inbred lines because of the relatively limited populations of *X. hortorum* pv. *carotae* recovered from the foliage of these PIs, combined with relatively limited bacterial blight severity ratings (Table 2.1). In contrast, PIs 226636, 234621, 277710, 390893, and 390887 were selected as putative susceptible PIs for backcrossing to the two inbreds because of the relatively greater populations of *X. hortorum* pv. *carotae* recovered from the foliage (Table 2.1). However, as stated above, none of the roots of these PIs survived the vernalization protocol, as a result of *A. radicina* infection and/or desiccation.

2013 resistance screening. Bacterial blight symptoms were observed 6 wpi on at least one, and typically all, inoculated plants among the two carrot inbred lines, 13 wild relatives, 15 PI lines, and 12 cultivars in the 2013 screening. Bacterial blight symptoms were not observed on most plants of PI 418967 and Nunhems 2-1 when evaluated 2, 3, and 4 wpi. Symptoms were observed on two of the 5 blocks of non-inoculated A2566 plants at both 3 and 4 wpi, and one of

the 5 replications of non-inoculated A0493 plants 4 wpi. By 6 wpi, 3 of the 25 non-inoculated male-sterile inbred plants of each of A2566 and A0493 had 3% foliage blighted, while the remaining 22 plants of each line were asymptomatic. As in the 2012 resistance screening, severity of foliar disease symptoms varied among the carrot genotypes evaluated (Table 2.4 and Fig. 2.2B). Additionally, slightly different symptoms were observed for some of the PIs and cultivars that were not observed in 2012. In particular, lesions on plants of Ames 26384 were chlorotic, without water-soaking, and had relatively distinct margins, unlike lesions on most of the lines and cultivars tested (Fig. 2.1F).

Disease severity measured 2, 3, 4, and 6 wpi varied among the PIs and cultivars evaluated in 2013 (Fig. 2.2B). For all 42 PIs and cultivars evaluated in 2013, disease ratings 2 wpi averaged 1.9 ± 0.1% and ranged from 0 to 10% foliage blighted, disease ratings 3 wpi averaged 4.8 ± 0.3% and ranged from 0 to 20%, and disease ratings 4 wpi averaged 7.5 ± 0.4% and ranged from 0 to 30% (Table 2.4). By 6 wpi, foliar disease ratings ranged from 0 to 90% and averaged 11.8 ± 0.4%. The eight putatively resistant PIs selected from the 2012 screening together averaged foliar severity ratings of 0.6 ± 0.2, 2.3 ± 0.3, 4.9 ± 0.4, and 5.0 ± 0.5% (range of 0 to 3, 0 to 10, 0 to 10, and 0 to 50%, respectively) when rated 2, 3, 4, and 6 wpi, respectively (Fig. 2.3B). The five putative susceptible PIs selected from 2012 together averaged foliar severity ratings of 4.0 ± 0.6, 10.4 ± 1.2, 15.0 ± 1.5, and 23.4 ± 1.9% bacterial blight (range of 0 to 10, 3 to 20, 3 to 30, and 0 to 90%, respectively) when observed 2, 3, 4, and 6 wpi, respectively. The 12 commercial cultivars tested in 2013 averaged 1.8 ± 0.2, 4.7 ± 0.4, 7.4 ± 0.6, and 13.5 ± 0.6% bacterial blight severity (0 to 5, 0 to 15, 0 to 20, and 0 to 50%, respectively) when evaluated 2, 3, 4, and 6 wpi, respectively. The 13 carrot wild relatives averaged 2.0 ± 0.2, 4.5 ± 0.4, 6.3 ± 0.5, and 9.8 ± 0.6% (0 to 5, 0 to 10, 0 to 20, and 0 to 60%, respectively) at 2, 3, 4, and 6 wpi,

respectively. The two male-sterile inbred lines averaged 2.4 ± 0.4, 4.7 ± 0.7, 7.5 ± 1.2, and 11.7 ± 1.2% foliage blighted (0 to 3, 3 to 10, 3 to 15, and 3 to 40%, respectively) 2, 3, 4, and 6 wpi, respectively.

Spearman's correlation coefficients of disease ratings 2 vs. 3 vs. 4 vs. 6 wpi were highly significant ($P < 0.0001$) for all 85 lines and cultivars tested (r ranged from 0.4768 to 0.8191) (Table 2.6). Likewise, Spearman's correlation coefficients of foliar disease ratings 2, 3, 4, and 6 wpi were highly significant ($P < 0.0001$) for the 15 PIs (r ranged from 0.5750 to 0.8405), and were also significant ($P < 0.0001$ to 0.0209) for the 12 cultivars (r ranged from 0.2976 to 0.8363) (Table 2.6). Foliar ratings for the 13 carrot wild relatives also were correlated highly significantly, with r ranging from 0.4044 to 0.8008 ($P < 0.0001$ to 0.0020) (Table 2.6). Friedman's rank test of $\log_{10}$ CFU/g dry foliage and disease severity ratings 6 wpi data showed a highly significant effect of carrot line/cultivar on both foliar severity ratings and population of the pathogen detected ($P < 0.0001$).

X. hortorum pv. *carotae* was recovered from every inoculated plant of the carrot inbred lines, wild relatives, PI lines, and cultivars tested in 2013. The pathogen was not detected on 8 of 50 non-inoculated male-sterile inbred plants. For 18 of the 50 control plants, the pathogen averaged ≤ $\log_{10}$ 4.000 CFU/g dry foliage, and for the 24 remaining control plants, the population of the pathogen recovered ranged from $\log_{10}$ 4.059 to $\log_{10}$ 8.110 CFU/g dry foliage. Population of the pathogen detected on inoculated plants 6 wpi for all lines and cultivars averaged $\log_{10}$ 9.132 ± 0.043 CFU/g dry foliage ($\log_{10}$ 4.690 to $\log_{10}$ 11.113 CFU/g), with an interquartile range of $\log_{10}$ 8.468 to $\log_{10}$ 10.092 CFU/g (Table 2.5 and Fig. 2.3B). Similar to the 2012 screening, disease symptoms did not appear on foliage until *X. hortorum* pv. *carotae* populations were > $\log_{10}$ 6.000 CFU/g dry foliage, with a distinct trend of a diminishing rate of

increase in bacterial populations as severity of symptoms increased (Fig. 2.2B). Foliar disease ratings 2, 3, 4, and 6 wpi for all lines and cultivars tested were highly significantly correlated ($P < 0.0001$) with *X. hortorum* pv. *carotae* population detected 6 wpi, with r = 0.4550, 0.5606, 0.5660, and 0.6162, respectively (Table 2.6). The eight resistant PIs averaged $\log_{10}$ 7.817 ± 0.102 CFU/g dry foliage ($\log_{10}$ 4.794 to $\log_{10}$ 10.579 CFU/g) 6 wpi, with an interquartile range of $\log_{10}$ 6.744 to $\log_{10}$ 9.068 CFU/g (Fig. 2.3B). In contrast, the five susceptible PIs selected averaged $\log_{10}$ 9.538 ± 0.081 CFU/g ($\log_{10}$ 5.163 to $\log_{10}$ 11.052 CFU/g) with an interquartile range of $\log_{10}$ 9.234 to $\log_{10}$ 9.979 CFU/g (Fig. 2.3B). Cultivars and carrot wild relatives averaged $\log_{10}$ 9.577 ± 0.068 and $\log_{10}$ 9.223 ± 0.069 CFU/g, respectively ($\log_{10}$ 5.748 to $\log_{10}$ 10.979 and $\log_{10}$ 6.366 to $\log_{10}$ 11.113 CFU/g, respectively), with interquartile ranges of $\log_{10}$ 9.273 to $\log_{10}$ 10.320 and $\log_{10}$ 8.523 to $\log_{10}$ 10.093 CFU/g, respectively (Fig. 2.3B).

Bacterial population recovered vs. foliar disease severity measured 2, 3, 4, and 6 wpi for all 15 PIs evaluated in 2013 were all highly significantly correlated ($P < 0.0001$) with r = 0.6162, 0.7399, 0.6809, and 0.5867, respectively (Table 2.6). Spearman's correlation coefficients for *X. hortorum* pv. *carotae* population recovered vs. foliar bacterial blight severity measured 2, 3, 4, and 6 wpi for the 12 cultivars tested in 2013 also were highly significantly correlated, with r ranging from 0.2896 to 0.6540 ($P < 0.0001$ to 0.0021) (Table 2.6). Similarly, Spearman's correlation coefficients for bacterial population vs. disease severity rated 2, 3, 4, and 6 wpi for the 12 carrot wild relatives were significant, with r ranging from 0.2810 ($P = 0.0360$) to 0.4937 ($P < 0.0001$) (Table 2.6). Spearman's correlation coefficients for *X. hortorum* pv. *carotae* population vs. disease severity ratings 2, 3, and 4 wpi for the two male-sterile inbred lines were not significant (*data not shown*), but the correlation was significant for the 6 wpi rating (r = 0.5742 at $P < 0.0001$).

The putative resistant and susceptible PIs selected from the 2012 screening had similar reactions to *X. hortorum* pv. *carotae* in the 2013 screening, although the average disease severity rating for some PIs was greater in 2013 than in 2012. In 2013, plants of the cultivar Nunhems 2-1 had disease ratings that averaged 0.0, 0.0, and 0.4% at 2, 3, and 4 wpi, respectively (Table 2.4). This cultivar had the least severe foliar blight measured 6 wpi, with an average 0.4 ± 0.2% foliage blighted (range of 0 to 3%), that was similar statistically to the ratings of PIs 418967 and 432906 (Table 2.4). Nunhems 2-1 had the second smallest mean population of *X. hortorum* pv. *carotae* recovered from the foliage, $\log_{10}$ 6.873 ± 0.120 CFU/g (range of $\log_{10}$ 5.748 to $\log_{10}$ 7.879 CFU/g), which was similar statistically to that of PIs 263601, 418967, 432905, 432906, and Ames 7674 (Table 2.5). As in the 2012 screening, PI 418967 had the least *X. hortorum* pv. *carotae* recovered from foliage, $\log_{10}$ 6.250 ± 0.164 CFU/g dry foliage (range of $\log_{10}$ 4.796 to $\log_{10}$ 7.778 CFU/g) which was similar statistically to that of PIs 432905, 432906 and Nunhems 2-1 (Table 2.5). PI 418967 averaged 0.0, 0.0, and 1.1% foliar disease severity 2, 3, and 4 wpi, respectively (Table 2.4). This PI had the second least severe foliar blight 6 wpi, 1.8 ± 0.4% (range of 0 to 5%), which was similar statistically to that of PIs 436674, Ames 7674, CEC1 WA, and Nunhems 2-1 (Table 2.4). In the 2013 evaluation, male-sterile inbred lines A2566 and A0493 averaged $\log_{10}$ 9.914 ± 0.081 and $\log_{10}$ 9.867 ± 0.105 CFU/g dry foliage (range of $\log_{10}$ 8.921 to $\log_{10}$ 10.650 and $\log_{10}$ 8.848 to $\log_{10}$ 10.815 CFU/g, respectively) with interquartile ranges of $\log_{10}$ 9.651 to $\log_{10}$ 10.146 and $\log_{10}$ 9.481 to $\log_{10}$ 10.307 CFU/g, respectively (Fig. 2.3 and 2.4).

Among the resistant PIs selected, 263601 had the narrowest interquartile range in 2013, $\log_{10}$ 7.132 to $\log_{10}$ 8.001 CFU/g, and averaged $\log_{10}$ 7.613 ± 0.185 CFU/g; whereas PI 163238 had the broadest interquartile range of $\log_{10}$ 6.488 to $\log_{10}$ 9.168 CFU/g dry foliage, and averaged

$\log_{10}$ 8.003 ± 0.327 CFU/g (Fig. 2.4A). The other six resistant selections, PIs 176969, 177381, 432905, 432906, and 436674 averaged $\log_{10}$ 9.184 ± 0.166, $\log_{10}$ 9.314 ± 0.207, $\log_{10}$ 7.160 ± 0.191, $\log_{10}$ 7.157 ± 0.208, and $\log_{10}$ 8.167 ± 0.210 CFU/g, respectively, with interquartile ranges of $\log_{10}$ 8.628 to $\log_{10}$ 9.754, $\log_{10}$ 8.676 to $\log_{10}$ 10.053, $\log_{10}$ 6.688 to $\log_{10}$ 7.618, $\log_{10}$ 6.429 to $\log_{10}$ 7.603, and $\log_{10}$ 7.278 to $\log_{10}$ 9.003 CFU/g, respectively (Fig. 2.4A). The other four 2012 resistant selections, PI 226636, 234621, 277710, and 390893, averaged $\log_{10}$ 9.528 ± 0.111 CFU/g, $\log_{10}$ 9.622 ± 0.066 CFU/g, $\log_{10}$ 9.055 ± 0.296 CFU/g, and $\log_{10}$ 10.001 ± 0.138 CFU/g, respectively (ranges of $\log_{10}$ 8.392 to $\log_{10}$ 10.603, $\log_{10}$ 8.860 to $\log_{10}$ 10.333, $\log_{10}$ 5.163 to $\log_{10}$ 10.348, and $\log_{10}$ 8.468 to $\log_{10}$ 11.052 CFU/g, respectively) (Fig. 2.4B).

Among the susceptible PI selections, disease severity ratings 2, 3, and 4 wpi for PI 390887 averaged 7.4, 19.0, and 25.0%, respectively, and ranged from 5 to 10, 15 to 20, and 20 to 30%, respectively (Table 2.4). This PI had the greatest disease severity rating 6 wpi, with 44.8 ± 4.2% foliage blighted (range of 5 to 80%), which was similar statistically to PI 390893 and Nunhems 2-2 (Table 2.4). However, PI 390887 had a similar mean population of *X. hortorum* pv. *carotae* recovered from the foliage ($\log_{10}$ 9.481 ± 0.172 CFU/g, with a range of $\log_{10}$ 7.068 to $\log_{10}$ 10.475 CFU/g) to that of 16 other PIs and cultivars evaluated in 2013 (Table 2.5). Nunhems 2-2 had the greatest mean bacterial population detected on the foliage, $\log_{10}$ 10.407 ± 0.066 CFU/g (range of $\log_{10}$ 9.649 to $\log_{10}$ 10.974 CFU/g), which was similar statistically to that of Ames 26381, Nunhems 1-3, Tendersnax, Nunhems 2-3, and Nunhems 2-4 (Table 2.5). Foliar bacterial blight ratings 2, 3, and 4 wpi for Nunhems 2-2 averaged 3.8, 9.4, and 14.0%, respectively (range of 3 to 5, 7 to 10, and 10 to 20% severity, respectively) (Table 2.4). This cultivar was among the entries with the most severe foliar ratings 6 wpi, 24.8 ± 1.5% foliage blighted (10 to 40%) for individual plants, which was similar statistically to the foliar ratings of

PIs 234621, 390893, 390887, and Nunhems 2-3 (Table 2.4). Of the five susceptible PIs selected, PI 234621 had the narrowest interquartile range of $\log_{10}$ 9.425 to $\log_{10}$ 9.896 CFU/g dry foliage, with an average $\log_{10}$ 9.622 ± 0.066 CFU/g; while PI 390887 had the broadest interquartile range of $\log_{10}$ 9.029 to $\log_{10}$ 10.053 CFU/g dry foliage, with an average $\log_{10}$ 9.481 ± 0.172 CFU/g (Fig. 2.4B).

Although not selected as resistant or susceptible from the 2012 screening, PI 357984 was included in the 2013 screening because the foliage developed abnormally in the 2012 trial from unknown factors, confounding bacterial blight severity ratings. PI 357984 had more severe bacterial blight symptoms and greater populations of *X. hortorum* pv. *carotae* recovered from the foliage than some of the susceptible selections from the 2012 screening. Disease severity ratings 2, 3, 4, and 6 wpi averaged 1.8, 4.6, 6.4, and 11.8 ± 1.6% foliage blighted, respectively (range of 0 to 3, 3 to 7, 3 to 10, and 3 to 30%, respectively), and the bacterial population recovered from the foliage 6 wpi averaged $\log_{10}$ 9.730 ± 0.076 CFU/g dry foliage ($\log_{10}$ 9.017 to $\log_{10}$ 10.386 CFU/g) (Tables 2.4 and 2.5).

Overall, PIs 418967, 432905, and 432906 were the most resistant PIs in the 2013 screening; and PIs 357984 and 390893, and Ames 26381, 26384, and 30198 were the most susceptible. Plants of these PIs and carrot wild relatives were crossed in a greenhouse in spring and summer 2014 with male-sterile inbred lines A2566 and A0493, based on the numbers of plants of each line that survived vernalization and transplanting. One cross was made of PI 418967 to each of A2566 and A0493; two crosses of PI 432905 to each of A2566 and A0493; two crosses of PI 432906 to A2566 and four crosses to A0493; two crosses of PI 357984 to each of A2566 and A0493; two crosses of PI 390893 to A2566 and one cross to A0493; three crosses

of Ames 26381 to each of A2566 and A0493; three crosses of Ames 26384 to each of A2566 and A0493; and one cross of Ames 30198 to A2566.

2.4. Discussion

To our knowledge, this is the first extensive public evaluation of PIs from the USDA NPGS carrot germplasm collection for resistance to *X. hortorum* pv. *carotae*. Potential resistance was identified in five PI lines (263601, 418967, 432905, and 432906) and one carrot wild relative (Ames 7674). Symptoms of bacterial blight were observed on almost all inoculated plants of 103 carrot lines and cultivars tested in the 2012 and 2013 *X. hortorum* pv. *carotae* carrot resistance screenings in this study. Additionally, *X. hortorum* pv. *carotae* was recovered from every inoculated plant. The variable bacterial populations recovered from the foliage and the diverse responses of the entries to inoculation with the bacterium indicate that the reactions of carrot lines to *X. hortorum* pv. *carotae* likely is quantitative. Involvement of multiple genes in carrot reactions to *X. hortorum* pv. *carotae* is consistent with resistance detected in other plant species to various *Xanthomonas* spp. Four genes have been described in tomato and five genes in pepper that contribute to hypersensitive reactions of these plants to *X. vesicatoria, X. euvesicatoria, X. perforans,* and *X. gardneri* (Stall et al., 2009). Blight symptoms observed on resistant and susceptible pepper and tomato plants during resistance screenings were highly variable (Jones et al., 2002; Scott et al., 1995; Scott et al., 2001; Yang et al., 2005), as in the 2012 and 2013 carrot screenings for resistance to *X. hortorum* pv. *carotae* in this study. These similarities in response to *Xanthomonas* spp. suggest that carrot germplasm may have similar genetic mechanisms of reactions to *X. hortorum* pv. *carotae* that tomato and pepper have to *Xanthomonas* spp. pathogenic on those plants.

Based on disease severity ratings and pathogen populations detected on foliage in the 2013 screening in this study, carrot PIs 418967 and 432906 were comparable to Nunhems 2-1, a proprietary cultivar in development specifically for resistance to *X. hortorum* pv. *carotae*. Nunhems 2-1 had the least severe foliar blighting, although PI lines 418967 and 432906 had statistically similar foliar blighting. These two PIs, in addition to PIs 263601 and 432905 had significantly less *X. hortorum* pv. *carotae* recovered from the foliage than that of the remaining 11 commercial carrot cultivars tested. Plants of PI 418967 had the least mean *X. hortorum* pv. *carotae* population recovered from the foliage, although plants of Nunhems 2-1 and PIs 432905 and 432906 had statistically similar bacterial populations recovered. Inbred male-sterile lines A2566 and A0493 had less variable populations of *X. hortorum* pv. *carotae* recovered from foliage in the statistically more robust 2013 screening, compared to the resistant and susceptible PI selections, which is not unexpected given the PIs are likely quite heterogeneous genetically, even within individual PIs. However, PIs 226636 and 234621 also had narrow interquartile ranges of bacterial population recovered that were less than that of A0493. The more limited variation in bacterial populations recovered from foliage of A2566 and A0493 may reflect the genetic uniformity of these two lines gained from four generations of inbreeding with close sibling mating and 12 generations of inbreeding, respectively (Phil Simon, *personal communication*).

Foliage morphology varied among the carrot lines and cultivars evaluated in this study, from fused to lace-like leaflets, with the degree of pubescence varying from a complete lack of pubescence to abundant pubescence visible on the foliage. Foliage color varied from bright green to purple foliage among and even within PI lines (data not shown). The most susceptible PI line to *X. hortorum* pv. *carotae* in 2013, PI 390893, and two of the carrot wild relatives, Ames

26381 and 26384, had more pubescent foliage, averaging a score of 3.5 on a scale of 0 to 5, where 0 = no pubescence and 1 to 5 representing incrementally more pubescent foliage, compared to the most resistant PI lines in 2013 (PIs 418967, 432905, and 432906) which averaged a pubescence score of 1.0. However, in general, there was no apparent relationship between foliage type and population of *X. hortorum* pv. *carotae* recovered from the foliage or percentage foliage blighted (*data not shown*).

The highly significant correlation between *X. hortorum* pv. *carotae* populations and bacterial blight foliar ratings in both 2012 and 2013 confirmed that carrot bacterial blight symptoms do not develop until *X. hortorum* pv. *carotae* populations reach $>10^5$ to 10^6 CFU/g dry foliage. Foliage blighting ratings 6 wpi ranged from approximately 1 to 3% when *X. hortorum* pv. *carotae* populations recovered from foliage were $\leq 10^7$ CFU/g dry foliage, but symptoms 6 wpi ranged from 5 to 10% when bacterial populations recovered from foliage were approximately 10^7 to 10^9 CFU/g dry foliage, and were > 10% when bacterial populations ranged from 10^9 to 10^{10} CFU/g dry foliage, indicating a diminishing rate of increase in bacterial populations as severity of symptoms increased. The more robust sampling of five blocks of five plants/line in the 2013 screening enabled calculations of a more robust correlation between bacterial population and foliar disease ratings than sampling only five plants/line in the 2012 screening ($r = 0.5183$ at $P < 0.0001$ in 2012 vs. $r = 0.6162$ at $P < 0.0001$ in 2013).

Based on the correlation coefficients calculated for foliar disease ratings vs. population of *X. hortorum* pv. *carotae* detected on the foliage, evaluating disease severity 4 wpi for a group of plants/line was almost as effective at predicting the bacterial pathogen population on foliage as disease severity ratings done 6 wpi on individual plants. The former is more cost-effective for evaluating large numbers of plants, e.g., in preliminary bacterial blight resistance screenings,

enabling highly susceptible lines or cultivars to be identified readily without the more resource and labor-intensive protocol needed for bacterial population quantification on individual plants. However, adequate replication of entries is important for accurate assessment of the reaction of each entry or line to *X. hortorum* pv. carotae. For example, the 2013 screening enabled carrot lines and cultivars with limited bacterial growth on the foliage to be identified in a more robust manner than the 2012 screening. PI lines 176969 and 177381, with the third and eighth least *X. hortorum* pv. *carotae* recovered from foliage in 2012, respectively, may have been portrayed inaccurately as resistant because of the limited sample size and lack of true randomization of treatments in the 2012 greenhouse screening as a result of limited space to accommodate the number of lines screened, since these lines had the fifteenth and twentieth least *X. hortorum* pv. *carotae* recovered from foliage in the 2013 screening, respectively. However, all the highly susceptible selections from the 2012 screening were similarly susceptible to *X. hortorum* pv. *carotae* in the 2013 screening.

In 2012, one rack of non-inoculated control plants of the male-sterile inbred line A2566 had relatively large populations of *X. hortorum* pv. *carotae* recovered from the foliage ($> 10^7$ CFU/g dry foliage) of three of the five plants tested. In 2013, populations $> 10^7$ CFU/g dry foliage were recovered from the plants of only one non-inoculated replicate of control plants of both A2566 and A0493. Detection of the pathogen on these control plants demonstrated that some spread of *X. hortorum* pv. *carotae* occurred among entries in the trial, probably as a result of contact between plants of adjacent entries, movement of the bacterium during handling of the plants, and/or splash dispersal from rain leaking through seams of several adjacent roof panes of the greenhouse during rainy periods in each trial. However, the majority of non-inoculated control plants were not infected with *X. hortorum* pv. *carotae* in either trial, indicating that cross-

contamination is unlikely to have influenced the results adversely, particularly in the 2013 screening when all treatments were replicated five times, with five plants/replicate, and randomized.

Assays for detection of *X. hortorum* pv. *carotae* on carrot seed of the PI lines were not carried out because of the very small amount of carrot seed that could be ordered from the USDA NPGS (200 seeds/line). Although it is possible that *X. hortorum* pv. *carotae* inoculum could have been present on the PI seed planted, bacterial blight symptoms were not observed on carrot plants prior to inoculation with *X. hortorum* pv. *carotae* in each of the 2012 and 2013 screenings, and symptoms of bacterial blight did not develop until 2 to 3 wpi in both screenings. Therefore, it is unlikely that seedborne inoculum affected the results of these trials.

For 7 of the 15 carrot wild relatives assayed in 2013, > 20% of the plants senesced before adequate disease severity ratings and *X. hortorum* pv. *carotae* populations could be determined. All plants of the wild relatives Ames 29107 and Ames 29098 had senesced completely before sampling occurred for *X. hortorum* pv. *carotae* quantification. The carrot wild relatives Ames 7674 and SS10 OR were among the 10 lines and cultivars with the least average $\log_{10}$ CFU/g dry foliage, but only 13 and 10 plants were assayed, respectively, because of the annual and ephemeral native of these lines. It will be necessary to inoculate and evaluate such ephemeral *Daucus* spp. earlier than in this trial (10 weeks after planting and 6 wpi, respectively) to assess effectively the response of such lines to *X. hortorum* pv. *carotae*, i.e., Ames 7674, 30214, 29107, and 29098, RDC 5 WI, SS10 OR, and SS10 WI.

In conclusion, this study identified PI lines that show different levels of susceptibility or resistance to *X. hortorum* pv. *carotae*, and illustrates that the PI collection offers a public source of resistance for carrot breeders to develop bacterial blight-resistant cultivars. In comparison to

the only resistant carrot cultivar evaluated in this trial, Nunhems 2-1, PIs 263601, 418967, 432905, and 432906, and carrot wild relative Ames 7674 had equivalent levels of resistance to *X. hortorum* pv. *carotae*, whether measured as pathogen population recovered from the foliage or percentage foliage blighted in the 2013 screening. The resistance identified in these PI lines could be used to develop resistant cultivars by backcrossing the resistance into commercially acceptable genetic backgrounds (e.g., as done by Ellis, 1999 for carrot rust fly resistance). Each of the 12 carrot cultivars evaluated in this study, except Nunhems 2-1, was moderately to highly susceptible to *X. hortorum* pv. *carotae*, developing bacterial blight symptoms that averaged ≥ 4.4 and 7.5% foliage blighted in 2012 and 2013, respectively, and *X. hortorum* pv. *carotae* populations of ≥ $\log_{10}$ 7.443 and $\log_{10}$ 8.283 CFU/g dry foliage in 2012 and 2013, respectively. Carrot PI lines and wild relatives (e.g., PIs 390887 and 390893, and carrot wild relatives Ames 26381, 26384, and 30198) were among the most susceptible to *X. hortorum* pv. *carotae*. These susceptible lines could be used along with the resistant PIs to determine the inheritance of resistance and susceptibility to *X. hortorum* pv. *carotae* in carrot (e.g., as done by Scott et al., 2001 for resistance in tomato to bacterial spot caused by *X. campestris* pv. *vesicatoria*). Knowledge of the nature of inheritance of resistance to *X. hortorum* pv. *carotae* will help breed for bacterial blight resistance more efficiently. The development of *X. hortorum* pv. *carotae* resistant carrot cultivars will give the carrot seed industry and carrot growers additional options for management of the carrot bacterial blight pathogen.

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Table 2.1. 2012 screening of carrot lines and cultivars for resistance to *Xanthomonas hortorum* pv. *carotae*[a]

		Foliar disease severity (%)							
		4	5	6 wpi[d]			Log_{10} (CFU/g dry foliage)[e]		
Line/cultivar	Source[b]	wpi[c]	wpi[c]	$\bar{x} \pm SE$	Range	Rank	$\bar{x} \pm SE$	Range	Rank
Daucus carota									
PI 163238	USDA NPGS	5	5	8.0 ± 1.4	3 - 10	53 e-w	7.866 ± 0.851	4.842 - 9.568	11 a-i
PI 164136	USDA NPGS	20	20	6.2 ± 1.6	3 - 10	26 a-p	9.447 ± 0.257	8.714 - 10.234	42 h-z
PI 169482	USDA NPGS	5	5	8.0 ± 1.2	5 - 10	56 f-y	9.521 ± 0.248	8.646 - 9.976	49 m-aa
PI 169488	USDA NPGS	5	5	3.8 ± 0.5	3 - 5	4 a-d	9.189 ± 0.343	8.547 - 10.363	30 c-u
PI 174206	USDA NPGS	0	10	9.0 ± 2.9	5 - 20	51 d-w	9.606 ± 0.203	9.176 - 10.368	50 n-bb
PI 174828	USDA NPGS	5	5	4.6 ± 0.7	3 - 7	13 a-j	8.470 ± 0.508	7.162 - 9.313	16 a-m
PI 175132	USDA NPGS	20	20	8.6 ± 3.1	3 - 20	37 b-t	9.777 ± 0.238	9.125 - 10.222	65 r-dd
PI 175715	USDA NPGS	5	5	4.0 ± 1.2	0 - 7	6 a-e	8.835 ± 0.189	8.523 - 9.430	15 a-l
PI 175716	USDA NPGS	5	5	5.6 ± 2.0	0 - 10	23 a-n	9.265 ± 0.305	8.696 - 10.260	40 f-y
PI 175718	USDA NPGS	10	10	6.0 ± 1.2	3 - 10	22 a-n	9.008 ± 0.165	8.598 - 9.425	19 a-p
PI 175719	USDA NPGS	5	5	15.8 ± 8.6	5 - 50	60 h-y	9.471 ± 0.299	8.418 - 10.184	46 k-aa
PI 176557	USDA NPGS	5	5	5.2 ± 1.3	3 - 10	16 a-j	9.287 ± 0.086	9.171 - 9.620	31 d-v
PI 176558	USDA NPGS	5	10	7.8 ± 1.0	5 - 10	49 d-u	9.369 ± 0.228	8.968 - 10.187	37 f-x
PI 176559	USDA NPGS	5	5	13.0 ± 3.0	5 - 20	73 q-z	8.862 ± 0.272	8.009 - 9.611	17 a-n
PI 176969	USDA NPGS	0	0	2.0 ± 1.4	0 - 7	1 a	7.319 ± 0.621	5.280 - 8.651	3 ab
PI 177381	USDA NPGS	5	5	3.0 ± 1.4	0 - 7	2 ab	8.456 ± 0.356	7.676 - 9.639	8 a-g
PI 180834	USDA NPGS	5	5	4.2 ± 0.5	3 - 5	8 a-g	9.495 ± 0.055	9.372 - 9.664	43 h-aa
PI 181052	USDA NPGS	10	10	6.8 ± 0.9	5 - 10	32 b-s	9.167 ± 0.293	8.320 - 10.119	26 c-t
PI 181766	USDA NPGS	5	5	9.0 ± 3.0	3 - 20	41 c-t	9.324 ± 0.234	8.510 - 9.905	36 f-x
PI 181767	USDA NPGS	5	5	5.0 ± 1.7	0 - 10	15 a-j	9.209 ± 0.194	8.757 - 9.776	25 c-s
PI 182204	USDA NPGS	5	5	8.0 ± 3.1	3 - 20	30 a-q	9.788 ± 0.183	9.215 - 10.158	68 t-dd
PI 182207	USDA NPGS	5	5	5.8 ± 1.7	0 - 10	24 a-n	9.606 ± 0.115	9.171 - 9.851	52 p-bb
PI 183401	USDA NPGS	10	10	10.0 ± 2.8	3 - 20	61 h-y	9.900 ± 0.207	9.181 - 10.395	73 w-dd
PI 187237	USDA NPGS	0	0	5.8 ± 0.5	5 - 7	19 a-m	9.322 ± 0.257	8.539 - 10.080	32 e-w
PI 207480	USDA NPGS	10	20	14.0 ± 4.0	10 - 30	78 t-z	9.777 ± 0.065	9.579 - 9.941	66 s-dd
PI 220014	USDA NPGS	10	10	5.4 ± 0.7	3 - 7	17 a-k	9.141 ± 0.226	8.454 - 9.751	21 b-r
PI 222723	USDA NPGS	5	5	9.0 ± 3.0	3 - 20	45 c-u	9.297 ± 0.165	9.010 - 9.721	33 e-w
PI 223360	USDA NPGS	10	10	4.6 ± 0.7	3 - 7	11 a-i	9.474 ± 0.447	8.174 - 10.974	44 i-aa
PI 223777	USDA NPGS	20	20	9.0 ± 3.0	3 - 20	39 c-t	9.802 ± 0.331	8.892 - 10.911	60 q-dd

Table 2.1. *continued…*

Line/cultivar	Source[b]	Foliar disease severity (%)					Log_{10} (CFU/g dry foliage)[e]		
		4 wpi[c]	5 wpi[c]	6 wpi[d] $\bar{X} \pm SE$	6 wpi[d] Range	6 wpi[d] Rank	$\bar{X} \pm SE$	Range	Rank
PI 226636	USDA NPGS	20	20	12.4 ± 3.2	5 - 20	65 l-z	10.465 ± 0.152	10.004 - 10.871	85 dd
PI 234621	USDA NPGS	30	30	16.0 ± 2.4	10 - 20	82 v-z	10.192 ± 0.017	10.134 - 10.241	83 bb-dd
PI 249535	USDA NPGS	5	5	6.0 ± 1.9	0 - 10	28 a-q	8.546 ± 0.366	7.444 - 9.534	13 a-j
PI 251228	USDA NPGS	10	10	7.4 ± 1.1	5 - 10	46 d-u	8.966 ± 0.557	7.436 - 10.280	39 f-y
PI 261614	USDA NPGS	10	20	12.4 ± 3.2	5 - 20	64 k-z	9.941 ± 0.206	9.522 - 10.465	75 x-dd
PI 263601	USDA NPGS	0	0	3.2 ± 0.9	0 - 5	3 a-c	6.799 ± 0.904	4.140 - 9.517	4 a-c
PI 264236	USDA NPGS	5	10	8.0 ± 1.2	5 - 10	57 f-y	9.667 ± 0.171	9.129 - 10.188	54 q-cc
PI 264237	USDA NPGS	5	10	9.8 ± 2.7	5 - 20	59 g-y	9.262 ± 0.171	8.827 - 9.875	27 c-t
PI 264543	USDA NPGS	5	5	6.6 ± 1.4	3 - 10	33 b-s	8.793 ± 0.566	7.725 - 10.461	23 c-s
PI 271471	USDA NPGS	5	5	13.0 ± 4.4	5 - 30	69 n-z	9.700 ± 0.372	8.362 - 10.317	62 r-dd
PI 274909	USDA NPGS	10	10	15.0 ± 4.5	5 - 30	75 r-z	9.793 ± 0.274	8.785 - 10.409	69 t-dd
PI 277710	USDA NPGS	10	10	10.4 ± 4.1	0 - 20	50 d-v	10.091 ± 0.191	9.495 - 10.645	78 z-dd
PI 280706	USDA NPGS	0	0	5.6 ± 1.2	3 - 10	21 a-m	8.603 ± 0.321	7.695 - 9.476	12 a-i
PI 284773	USDA NPGS	10	20	27.4 ± 9.5	7 - 50	81 v-z	9.872 ± 0.060	9.690 - 10.000	71 v-dd
PI 288458	USDA NPGS	20	20	22.5 ± 6.3	10 - 40	85 x-z	9.804 ± 0.304	9.057 - 10.380	63 q-dd
PI 288461	USDA NPGS	20	20	8.8 ± 0.7	7 - 10	63 j-y	9.522 ± 0.428	8.685 - 11.142	41 g-y
PI 294084	USDA NPGS	10	10	12.0 ± 2.0	10 - 20	77 s-z	9.890 ± 0.065	9.662 - 10.056	74 w-dd
PI 326010	USDA NPGS	5	10	6.0 ± 1.2	3 - 10	27 a-p	9.270 ± 0.170	9.044 - 9.944	29 c-u
PI 326011	USDA NPGS	10	5	9.6 ± 2.9	3 - 20	58 g-y	9.704 ± 0.209	8.959 - 10.162	58 q-dd
PI 344072	USDA NPGS	10	10	6.8 ± 1.8	0 - 10	34 b-t	9.714 ± 0.079	9.428 - 9.912	56 q-dd
PI 344447	USDA NPGS	10	10	8.6 ± 3.1	3 - 20	40 c-t	9.918 ± 0.116	9.632 - 10.296	76 x-dd
PI 357984	USDA NPGS	0	0	11.0 ± 3.7	3 - 20	54 e-w	9.885 ± 0.109	9.569 - 10.217	72 w-dd
PI 390885	USDA NPGS	30	30	16.0 ± 2.4	10 - 20	83 w-z	10.070 ± 0.115	9.805 - 10.380	81 aa-dd
PI 390889	USDA NPGS	20	20	13.0 ± 5.4	0 - 30	62 i-y	9.742 ± 0.308	8.705 - 10.643	57 q-dd
PI 390893	USDA NPGS	10	10	20.4 ± 8.0	5 - 40	70 o-z	10.289 ± 0.207	9.517 - 10.760	84 bbdd
PI 390900	USDA NPGS	20	20	19.0 ± 6.8	5 - 40	76 r-z	10.086 ± 0.137	9.778 - 10.433	80 aa-dd
PI 390901	USDA NPGS	10	10	14.6 ± 6.5	3 - 40	66 l-z	9.398 ± 0.333	8.535 - 10.489	38 f-x
PI 418967	USDA NPGS	0	5	7.4 ± 1.1	5 - 10	47 d-u	6.398 ± 0.498	4.549 - 7.485	1 a
PI 430524	USDA NPGS	10	10	7.6 ± 3.5	0 - 20	31 b-r	9.191 ± 0.517	7.183 - 9.923	45 i-aa

Table 2.1. *continued…*

Line/cultivar	Source[b]	Foliar disease severity (%) 4 wpi[c]	5 wpi[c]	6 wpi[d] $\bar{x} \pm SE$	6 wpi[d] Range	6 wpi[d] Rank	Log_{10} (CFU/g dry foliage)[e] $\bar{x} \pm SE$	Log_{10} (CFU/g dry foliage)[e] Range	Log_{10} (CFU/g dry foliage)[e] Rank
D. carota subsp. *carota*									
PI 390887	USDA NPGS	20	20	22.8 ± 9.2	7 - 50	74 r-z	10.002 ± 0.505	8.849 - 11.370	67 s-dd
PI 478863	USDA NPGS	5	5	7.2 ± 1.7	3 - 10	42 c-t	9.375 ± 0.100	9.165 - 9.703	35 f-w
PI 478873	USDA NPGS	10	10	5.6 ± 1.2	3 - 10	20 a-m	8.361 ± 0.471	7.031 - 9.822	14 a-k
D. carota subsp. *commutatus*									
PI 478883	USDA NPGS	5	5	3.6 ± 1.2	0 - 7	5 a-d	9.085 ± 0.213	8.297 - 9.539	20 b-q
D. carota subsp. *sativus*									
PI 432900	USDA NPGS	0	5	15.4 ± 6.2	7 - 40	72 p-z	8.085 ± 0.505	7.239 - 10.061	9 a-h
PI 432905	USDA NPGS	0	0	3.6 ± 1.9	0 - 10	7 a-f	7.250 ± 0.222	6.414 - 7.585	2 a
PI 432906	USDA NPGS	5	5	4.6 ± 0.7	3 - 7	10 a-h	8.190 ± 0.338	7.335 - 9.268	5 a-d
PI 436674	USDA NPGS	0	5	8.0 ± 3.1	3 - 20	29 a-q	7.479 ± 0.803	5.830 - 9.533	10 a-i
A2566	USDA-ARS	7.5	7.5	6.0 ± 0.7	0 - 10	84 z	9.339 ± 0.168	7.816 - 10.949	82 ccdd
A0493	USDA-ARS	5	5	4.8 ± 1.0	0 - 20	79 yz	8.871 ± 0.160	6.803 - 9.824	79 bbcc
Nash's Nantes	Nash Huber	15	20	19.0 ± 4.0	5 - 30	80 u-z	9.861 ± 0.277	8.848 - 10.311	70 u-dd
Nash's Rumba	Nash Huber	20	20	11.0 ± 3.7	3 - 20	52 e-w	9.680 ± 0.324	8.740 - 10.716	53 q-bb
68017-1	Bejo Seeds	5	5	11.0 ± 2.4	5 - 20	67 m-z	9.943 ± 0.418	9.009 - 11.516	59 q-dd
Nelson	Bejo Seeds	5	5	8.6 ± 3.1	3 - 20	43 c-u	9.770 ± 0.192	9.302 - 10.450	61 r-dd
Napa	Bejo Seeds	5	5	4.4 ± 1.2	0 - 7	14 a-j	9.173 ± 0.213	8.574 - 9.709	24 c-s
Cupar	Bejo Seeds	10	10	5.0 ± 2.2	0 - 10	18 a-l	7.443 ± 0.763	5.420 - 9.707	6 a-e
Nerac	Bejo Seeds	5	5	6.0 ± 1.2	3 - 10	25 a-o	8.966 ± 0.099	8.736 - 9.243	18 a-o
Nunhems 1-1	Nunhems, USA	10	10	9.8 ± 2.7	5 - 20	55 e-x	9.634 ± 0.062	9.494 - 9.804	51 o-bb
Nunhems 1-2	Nunhems, USA	0	5	4.6 ± 0.7	3 - 7	9 a-h	8.389 ± 0.291	7.372 - 9.146	7 a-f
Nunhems 1-3	Nunhems, USA	5	10	7.0 ± 1.4	3 - 10	38 b-t	9.501 ± 0.242	8.860 - 10.111	47 k-aa
Nunhems 1-4	Nunhems, USA	5	5	9.0 ± 3.0	3 - 20	48 d-u	9.148 ± 0.400	7.972 - 10.175	28 c-u
Sugarsnax	Nunhems, USA	5	5	10.4 ± 5.2	0 - 30	44 c-u	9.360 ± 0.581	7.464 - 10.856	48 l-aa
Tendersnax	Nunhems, USA	5	5	8.0 ± 3.1	3 - 20	35 b-t	9.761 ± 0.307	9.075 - 10.717	55 q-dd
Enterprise	Monsanto Vegetable Seeds	10	10	13.0 ± 3.0	5 - 20	71 p-z	9.743 ± 0.225	8.913 - 10.272	64 r-dd
Propeel	Monsanto Vegetable Seeds	5	5	7.0 ± 1.4	3 - 10	36 b-t	9.939 ±0.137	9.607 - 10.306	77 y-dd

Table 2.1. *continued…*

Line/cultivar	Source[b]	Foliar disease severity (%)					Log_{10} (CFU/g dry foliage)[e]		
				6 wpi[d]					
		4 wpi[c]	5 wpi[c]	$\bar{x} \pm SE$	Range	Rank	$\bar{x} \pm SE$	Range	Rank
Triton	Sakata America	5	5	4.6 ± 0.7	3 - 7	12 a-i	9.167 ± 0.191	8.818 - 9.842	22 c-r
Chantenay Red Core	Sakata America	5	10	11.4 ± 2.2	7 - 20	68 m-z	9.224 ± 0.371	8.089 - 10.125	34 e-w

[a] The experiment was set up with up to 25 carrot plants/line inoculated with *X. hortorum* pv. *carotae* in November 2012, 63 days after planting the seed, as described in the main text.

[b] Carrot Plant Introduction (PI) lines, male-sterile inbred lines, and cultivars were obtained from the United States Department of Agriculture (USDA) National Plant Germplasm System (NPGS); Dr. Phillip Simon's USDA Agricultural Research Service (ARS) carrot breeding program at the University of Wisconsin in Madison, WI; Nash Huber in Sequim, WA; Bejo Seeds, Inc.; Nunhems USA, Inc.; Monsanto Vegetable Seeds, Inc.; and Sakata America, Inc.

[c] Mean percentage carrot foliage blighted 4 and 5 weeks post inoculation (wpi) for up to 25 carrot plants/line.

[d] Mean ± standard error ($\bar{x} \pm SE$), range, and rank of the mean percentage blighted foliage of each carrot line 6 wpi, measured for up to five plants/line. Only four plants were sampled from PI 288458, and 20 plants each were sampled from the male sterile inbreds, A2566 and A0493. Mean ranks were compared using Fisher's protected least significant difference (LSD). Mean ranks with at least one letter in common were not significantly different ($P \leq 0.05$).

[e] $\bar{x} \pm SE$, range, and ranked means of the log (CFU *X. hortorum* pv. *carotae*/g dry foliage) measured 6 wpi. Mean ranks were compared using Fisher's protected LSD. Mean ranks with at least one letter in common were not significantly different ($P \leq 0.05$).

Table 2.2. Spearman's correlation coefficient, r (and associated *P*-value), between foliar bacterial blight severity measured 4, 5, and 6 weeks post inoculation (wpi) vs. population of *Xanthomonas hortorum* pv. *carotae* quantified on carrot foliage 6 wpi in a 2012 greenhouse screening of 66 carrot Plant Introduction (PI) lines, 17 cultivars, and 2 inbred male-sterile lines

Carrot entries	Foliar disease severity (%)	Foliar disease severity (%)		Log_{10} (CFU *X. hortorum* pv. *carotae*/g dry foliage)[b]
		5 wpi[a]	6 wpi[a]	
All entries[c]	4 wpi[a]	0.8868 (< 0.0001)	0.4432 (< 0.0001)	0.5416 (< 0.0001)
	5 wpi	-	0.4976 (< 0.0001)	0.5036 (< 0.0001)
	6 wpi	-	-	0.5183 (< 0.0001)
PI lines[d]	4 wpi	0.8658 (< 0.0001)	0.4236 (< 0.0001)	0.6188 (< 0.0001)
	5 wpi	-	0.4498 (< 0.0001)	0.6004 (< 0.0001)
	6 wpi	-	-	0.5295 (< 0.0001)
Cultivars[e]	4 wpi	0.8105 (< 0.0001)	0.5902 (0.0126)	0.1986 (0.4449)
	5 wpi	-	0.6873 (0.0023)	0.1883 (0.4692)
	6 wpi	-	-	0.5635 (< 0.0001)

[a] Percentage carrot foliage blighted 4 and 5 wpi for up to 25 carrot plants/line, or 6 wpi for 5 carrot plants/line. Carrot foliage was inoculated with *X. hortorum* pv. *carotae* as described in the main text.

[b] Mean *X. hortorum* pv. *carotae* population detected on carrot foliage 6 wpi for five carrot plants/line, as described in the main text.

[c] N = 91 plants rated for percentage foliage blighted 4 and 5 wpi; N = 454 plants evaluated for percentage foliage blighted and *X. hortorum* pv. *carotae* population detected on carrot foliage 6 wpi.

[d] N = 66 plants rated for percentage foliage blighted 4 and 5 wpi; N = 329 plants evaluated for percentage foliage blighted and *X. hortorum* pv. *carotae* population detected on carrot foliage 6 wpi.

[e] N = 17 plants rated for percentage foliage blighted 4 and 5 wpi; N = 85 plants evaluated for percentage foliage blighted and *X. hortorum* pv. *carotae* population detected on carrot foliage 6 wpi.

Table 2.3. Line, species, and source of carrot wild relatives and cultivars evaluated in a 2013 greenhouse screening for resistance to *Xanthomonas hortorum* pv. *carotae*

Line/cultivar	Species	Source[a]
Ames 30198	*Daucus capillifolius*	USDA NPGS
Ames 30214	*D. capillifolius*	USDA NPGS
SS109 CA	*D. carota*	USDA-ARS
RDC 5 WI	*D. carota*	USDA-ARS
SS10 OR	*D. carota*	USDA-ARS
Ames 7674	*D. carota* subsp. *commutatus*	USDA NPGS
PI 652291	*D. carota* subsp. *commutatus*	USDA NPGS
Ames 26381	*D. carota* subsp. *fontanesii*	USDA NPGS
Ames 31193	*D. carota* subsp. *gadecaei*	USDA NPGS
Ames 26382	*D. carota* subsp. *gummifer*	USDA NPGS
Ames 26383	*D. carota* subsp. *gummifer*	USDA NPGS
Ames 26384	*D. carota* subsp. *gummifer*	USDA NPGS
CEC1 WA	*Daucus* sp.	Charles E. Christianson
Nunhems 2-1	*D. carota* subsp. *sativus*	Nunhems USA, Inc.
Nunhems 2-2	*D. carota* subsp. *sativus*	Nunhems USA, Inc.
Nunhems 2-3	*D. carota* subsp. *sativus*	Nunhems USA, Inc.
Nunhems 2-4	*D. carota* subsp. *sativus*	Nunhems USA, Inc.

[a] Seed was supplied by the United States Department of Agriculture (USDA) National Plant Germplasm System (NPGS); Dr. Phillip Simon's USDA Agricultural Research Service (ARS) program at the University of Wisconsin in Madison, WI; Charles Christianson in Bay View, WA (N48.479931°, W122.473065°) for wild carrot seed; and Nunhems USA, Inc. for the last four cultivars listed.

Table 2.4. 2013 Greenhouse screening of carrot lines or cultivars for resistance to *Xanthomonas hortorum* pv. *carotae*: Mean disease severity, range in disease severity 2, 3, 4, and 6 weeks post inoculation (wpi), and means comparison of ranked disease severity ratings 6 wpi[a]

	2 wpi[b]		3 wpi[b]		4 wpi[b]		6 wpi[c]		
Line/cultivar	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean ± SE**	**Range**	**Rank**
PI 163238	0.8	0 - 3	3.0	1 - 5	5.6	3 - 10	7.6 ± 1.8	0 - 40	15 e-k
PI 176969	1.4	0 - 3	4.0	1 - 10	7.2	3 - 10	4.3 ± 0.9	0 - 20	6 c-e
PI 177381	0.6	0 - 3	3.8	3 - 5	6.0	3 - 10	5.2 ± 0.9	0 - 20	10 d-g
PI 187237	0.0	0	2.2	0 - 5	5.6	0 - 10	6.2 ± 1.6	0 - 40	13 e-i
PI 226636	3.0	3	6.2	5 - 7	10.4	7 -15	7.6 ± 1.2	0 - 20	17 g-l
PI 234621	3.0	3	8.6	3 - 15	14.0	10 - 20	22.5 ± 3.0	3 - 60	39 st
PI 263601	1.2	0 - 3	1.6	0 - 3	5.0	3 - 7	5.8 ± 1.3	0 - 30	11 d-h
PI 277710	1.2	0 - 3	6.0	3 - 10	7.6	3 - 15	8.2 ± 1.9	0 - 40	16 f-l
PI 357984	1.8	0 - 3	4.6	3 - 7	6.4	3 - 10	11.8 ± 1.6	3 - 30	29 o-q
PI 390893	5.6	3 - 10	13.0	10 - 20	19.0	15 - 25	34.4 ± 4.0	10 - 90	41 u
PI 418967	0.0	0	0.0	0	1.2	0 - 3	1.8 ± 0.4	0 - 5	2 ab
PI 390887	7.4	5 - 10	19.0	15 - 20	25.0	20 - 30	44.8 ± 4.2	5 - 80	42 u
PI 432905	0.6	0 - 3	2.4	0 - 3	5.0	3 - 7	6.1 ± 2.0	0 - 50	8 de
PI 432906	0.0	0	1.8	0 - 3	4.6	3 - 5	1.9 ± 0.4	0 - 7	3 a-c
PI 436674	0.0	0	1.8	0 - 5	4.2	1 - 7	7.2 ± 2.3	0 - 50	12 d-i
A2566	2.4	0 - 3	6.0	3 - 10	10.4	7 - 15	13.0 ± 2.0	3 - 40	30 o-q
A0493	2.4	0 - 3	3.4	3 - 5	4.6	3 - 5	10.4 ± 1.3	3 - 30	27 m-p
Nash's Nantes	0.6	0 - 3	2.4	0 - 3	4.6	3 - 7	8.5 ± 1.0	3 - 20	22 k-o
Nash's Rumba	2.4	0 - 3	7.2	5 - 10	10.0	10	19.5 ± 3.0	3 - 50	36 rs
68017-1 Bejo	1.2	0 - 3	3.2	0 - 5	6.4	3 - 10	9.1 ± 1.1	0 - 20	23 l-o
Cupar	0.6	0 - 3	3.4	3 - 5	5.4	5 - 7	8.6 ± 1.6	3 - 30	20 j-n
Nunhems 1-2	2.4	0 - 3	3.4	3 - 5	4.2	3 - 5	7.5 ± 1.0	3 - 20	19 i-m
Nunhems 1-3	0.6	0 - 3	3.4	3 - 5	8.2	5 - 15	17.4 ± 1.9	3 - 30	35 rs
Tendersnax	1.2	0 - 3	3.6	0 - 7	6.2	3 - 7	14.3 ± 1.8	5 - 40	32 p-s
Triton	1.8	0 - 3	4.6	3 - 5	8.4	5 - 15	15.2 ± 1.5	3 - 30	34 q-s
Ames 30198	1.2	0 - 3	2.4	0 - 3	5.2	3 - 10	7.8 ± 1.1	0 - 20	18 h-m
Ames 30214	3.7	3 - 5	8.3	5 - 10	10.0	5 - 15	22.8 ± 5.0	3 - 50	33 p-s
SS109 CA	1.2	0 - 3	5.8	0 - 10	9.0	0 - 20	5.5 ± 0.7	0 - 10	14 d-j
RDC 5 WI	3.0	3	6.5	3 - 10	7.5	5 - 10	15.8 ± 5.7	3 - 40	25 k-r

Table 2.4. *continued…*

	2 wpi[b]		3 wpi[b]		4 wpi[b]		6 wpi[c]		
Line/cultivar	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean ± SE**	**Range**	**Rank**
SS10 OR	1.0	0 - 3	2.7	0 - 5	3.7	3 - 5	5.7 ± 1.6	3 - 20	7 b-f
Ames 7674	1.0	0 - 3	2.3	1 - 3	3.0	1 - 5	4.8 ± 0.9	0 - 10	5 b-e
PI 652291	2.4	0 - 3	5.0	3 - 7	7.0	7	11.9 ± 1.5	0 - 20	28 n-p
Ames 26381	2.4	0 - 3	5.2	3 - 10	7.2	5 - 10	19.1 ± 3.4	3 - 60	31 p-s
Ames 31193	0.0	0	2.6	1 - 5	3.8	3 - 5	5.0 ± 0.7	0 - 20	9 d-g
Ames 26382	3.4	3 - 5	3.4	3 - 5	4.6	3 - 5	9.2 ± 1.5	0 - 30	21 k-n
Ames 26383	3.4	3 - 5	5.4	3 - 7	6.6	5 - 7	10.8 ± 1.7	0 - 30	24 l-o
Ames 26384	4.2	3 - 5	8.4	5 - 10	11.0	10 - 15	11.8 ± 2.0	0 - 30	26 m-p
CEC1 WA	0.0	0	1.2	0 - 3	3.2	0 - 7	3.4 ± 0.4	0 - 7	4 b-d
Nunhems 2-1	0.0	0	0.0	0	0.4	0 - 1	0.4 ± 0.2	0 - 3	1 a
Nunhems 2-2	3.8	3 - 5	9.4	7 - 10	14.0	10 - 20	24.8 ± 1.5	10 - 40	40 tu
Nunhems 2-3	3.0	3	6.2	5 - 7	8.8	7 - 10	17.2 ± 1.5	7 - 30	38 st
Nunhems 2-4	3.8	3 - 5	9.8	7 - 15	12.4	7 - 15	19.0 ± 2.2	3 - 50	37 s

[a] The experiment was set up as a randomized complete block design with five carrot plants/block and five replicate blocks of all lines. Plants were inoculated with *X. hortorum* pv. *carotae* in July 2013, 65 days after planting the seed, as described in the main text.

[b] Mean and range of the percentage carrot foliage blighted 2, 3, and 4 wpi for up to 25 carrot plants/line.

[c] Mean ± standard error (SE), range, and rank of the percentage carrot foliage blighted 6 wpi for five carrot plants assayed/replication/line. Ranks for each line were compared using Fisher's protected least significant difference (LSD at $P \leq 0.05$). Lines with means separation letters in common were not significantly different in ranking.

Table 2.5. 2013 Greenhouse screening of carrot Plant Introduction (PI) lines, cultivars, and wild relatives for resistance to *Xanthomonas hortorum* pv. *carotae* based on population of the pathogen detected on the foliage 6 weeks post-inoculation (wpi)[a]

		Log_{10} (CFU/g dry foliage)		
Line/cultivar	**N[b]**	**Mean ± SE[c]**	**Range[d]**	**Rank[e]**
PI 163238	23	8.003 ± 0.327	5.587 - 10.400	10 d-f
PI 176969	21	9.184 ± 0.166	7.744 - 10.383	15 h-k
PI 177381	23	9.314 ± 0.207	7.105 - 10.579	20 j-m
PI 187237	23	8.914 ± 0.305	4.690 - 10.468	14 h-j
PI 226636	24	9.528 ± 0.111	8.392 - 10.603	30 j-n
PI 234621	25	9.622 ± 0.066	8.860 - 10.333	24 k-q
PI 263601	25	7.613 ± 0.185	5.444 - 9.647	5 b-d
PI 277710	23	9.055 ± 0.296	5.163 - 10.348	18 i-l
PI 357984	23	9.730 ± 0.076	9.017 - 10.386	27 m-r
PI 390893	23	10.001 ± 0.138	8.468 - 11.052	36 s-w
PI 418967	24	6.250 ± 0.164	4.796 - 7.778	1 a
PI 390887	24	9.481 ± 0.172	7.068 - 10.475	23 k-p
PI 432905	25	7.160 ± 0.191	5.213 - 9.356	3 a-c
PI 432906	25	7.157 ± 0.208	4.794 - 9.273	4 a-c
PI 436674	24	8.167 ± 0.210	6.432 - 9.611	8 de
A2566	25	9.914 ± 0.081	8.921 - 10.650	32 q-s
A0493	25	9.867 ± 0.105	8.848 - 10.815	30 o-s
Nash's Nantes	25	9.718 ± 0.155	7.631 - 10.979	28 m-s
Nash's Rumba	23	9.809 ± 0.133	8.066 - 10.526	31 p-s
68017-1 Bejo	25	9.648 ± 0.095	8.668 - 10.283	25 l-r
Cupar	24	8.283 ± 0.275	6.124 - 10.342	11 e-g
Nunhems 1-2	24	9.349 ± 0.094	8.467 - 10.131	17 h-k
Nunhems 1-3	25	10.262 ± 0.064	9.694 - 10.942	41 x
Tendersnax	25	10.259 ± 0.060	9.816 - 10.930	40 wx
Triton	25	9.912 ± 0.081	9.086 - 10.592	33 r-t
Ames 30198	22	9.814 ± 0.216	6.909 - 10.918	35 r-v
Ames 30214	11	9.550 ± 0.319	7.865 - 10.486	26 k-s
SS109 CA	19	9.721 ± 0.213	7.781 - 10.817	29 n-s
RDC 5 WI	6	9.404 ± 0.366	7.732 - 10.232	19 g-o
SS10 OR	10	8.159 ± 0.462	6.494 - 10.421	9 c-f

Table 2.5. *continued…*

Line/cultivar	N[b]	Log_{10} (CFU/g dry foliage)		
		Mean ± SE[c]	Range[d]	Rank[e]
Ames 7674	13	8.205 ± 0.230	6.972 - 9.424	6 b-e
PI 652291	23	9.591 ± 0.112	8.269 - 10.533	22 k-p
Ames 26381	25	10.136 ± 0.157	7.454 - 11.113	37 t-x
Ames 31193	25	8.857 ± 0.188	7.034 - 10.351	13 f-i
Ames 26382	25	8.801 ± 0.187	6.366 - 10.436	12 f-h
Ames 26383	25	9.097 ± 0.187	7.332 - 10.530	16 h-k
Ames 26384	22	9.843 ± 0.168	8.135 - 10.866	34 r-u
CEC1 WA	24	8.166 ± 0.169	6.393 - 9.507	7 c-e
Nunhems 2-1	25	6.873 ± 0.120	5.748 - 7.879	2 ab
Nunhems 2-2	25	10.407 ± 0.066	9.649 - 10.974	42 x
Nunhems 2-3	25	10.213 ± 0.085	9.128 - 10.890	39 v-x
Nunhems 2-4	25	10.145 ± 0.138	7.549 - 10.867	38 u-x

[a] Carrot Plant Introduction (PI) lines, cultivars, and wild relatives (see Tables 2.1 and 2.3) were inoculated with *X. hortorum* pv. *carotae* in a greenhouse in 2013, and the foliage of the plants tested to quantify the pathogen 6 wpi, as described in the main text.

[b] Number of plants assayed for *X. hortorum* pv. *carotae* 6 wpi.

[c] Mean ± standard error (SE) of *X. hortorum* pv. *carotae* population detected on the foliage 6 wpi for up to five replications of five plants/replication.

[d] Range in population of *X. hortorum* pv. *carotae* detected 6 wpi for up to five plants in each of five replications.

[e] Mean rank in *X. hortorum* pv. *carotae* population detected on the foliage 6 wpi for five plants sampled from each of five replications. Ranks for each line were compared using Fisher's protected least significant difference (LSD at $P \leq 0.05$). Lines with means separation letters in common were not significantly different.

Table 2.6. Spearman's correlation coefficient, r (and associated *P*-value), between foliar bacterial blight severity measured 2, 3, 4, and 6 weeks post inoculation (wpi) vs. population of *Xanthomonas hortorum* pv. *carotae* quantified on carrot foliage 6 wpi in a 2013 greenhouse screening of 15 carrot Plant Introduction (PI) line selections from the 2012 greenhouse screening, as well as 12 carrot cultivars, 2 inbred male-sterile lines, and 13 carrot wild relatives

Carrot entries	Foliar disease severity (%)	Foliar disease severity (%)[a]			Log_{10} (CFU *X. hortorum* pv. *carotae*/g dry foliage)[b]
		3 wpi	4 wpi	6 wpi	
All entries[c]	2 wpi[a]	0.6819 (< 0.0001)	0.5706 (< 0.0001)	0.4768 (< 0.0001)	0.4550 (< 0.0001)
	3 wpi	-	0.8191 (< 0.0001)	0.5334 (< 0.0001)	0.5606 (< 0.0001)
	4 wpi	-	-	0.5311 (< 0.0001)	0.5660 (< 0.0001)
	6 wpi	-	-	-	0.6162 (< 0.0001)
PI lines[d]	2 wpi	0.7468 (< 0.0001)	0.6919 (< 0.0001)	0.5750 (< 0.0001)	0.6162 (< 0.0001)
	3 wpi	-	0.8405 (< 0.0001)	0.6154 (< 0.0001)	0.7399 (< 0.0001)
	4 wpi	-	-	0.5830 (< 0.0001)	0.6809 (< 0.0001)
	6 wpi	-	-	-	0.5867 (< 0.0001)
Cultivars[e]	2 wpi	0.6636 (< 0.0001)	0.5748 (< 0.0001)	0.2976 (0.0209)	0.3896 (0.0021)
	3 wpi	-	0.8363 (< 0.0001)	0.5370 (< 0.0001)	0.5061 (< 0.0001)
	4 wpi	-	-	0.5729 (< 0.0001)	0.5341 (< 0.0001)
	6 wpi	-	-	-	0.6540 (< 0.0001)
Carrot wild relatives[f]	2 wpi	0.6152 (< 0.0001)	0.4952 (0.0001)	0.5056 (< 0.0001)	0.2810 (0.0360)
	3 wpi	-	0.8008 (< 0.0001)	0.4044 (0.0020)	0.3327 (0.0122)
	4 wpi	-	-	0.4657 (0.0003)	0.4533 (0.0005)
	6 wpi	-	-	-	0.4937 (< 0.0001)

[a] Percentage carrot foliage blighted 2, 3, and 4 wpi for up to five replicate sets of five plants/line, and 6 wpi for up to five replicate blocks of five carrot plants/line. Carrot foliage was inoculated with *X. hortorum* pv. *carotae* as described in the main text.

[b] *X. hortorum* pv. *carotae* population detected on carrot foliage 6 wpi of up to five replications of five carrot plants/line, as described in the main text.

[c] N = 201 entries rated 2, 3, and 4 wpi for percentage foliage blighted; and N = 951 plants evaluated for percentage foliage blighted and *X. hortorum* pv. *carotae* population detected on carrot foliage 6 wpi.

[d] N = 75 entries rated 2, 3, and 4 wpi for percentage foliage blighted; and N = 355 plants evaluated for percentage foliage blighted and *X. hortorum* pv. *carotae* population detected on carrot foliage 6 wpi.

[e] N = 60 entries rated 2, 3, and 4 wpi for percentage foliage blighted; and N = 296 plants evaluated for percentage foliage blighted and *X. hortorum* pv. *carotae* population detected on carrot foliage 6 wpi.

[f] N = 56 entries rated 2, 3, and 4 wpi for percentage foliage blighted; and N = 250 plants evaluated for percentage foliage blighted and *X. hortorum* pv. *carotae* population detected on carrot foliage 6 wpi.

Fig. 2.1. Symptoms of bacterial blight 4 to 5 weeks following inoculation of the foliage with *Xanthomonas hortorum* pv. *carotae* in greenhouse trials as described in the main text. Chlorotic, water-soaked lesions on the carrot cultivars A) Nunhems 2-2, B) 68017-1, and C) Nunhems 2-4; D) purple symptoms on Plant Introduction (PI) line 288458; E) purple, necrotic, and dry lesions on PI 226639; and F) chlorotic lesions with distinct margins on carrot wild relative Ames 26384. Nunhems 2-2 and 2-4 were obtained from Nunhems USA, Inc., cultivar 68017-1 from Bejo Seeds, Inc., and PIs 226639 and 288458 from the United States Department of Agriculture National Plant Germplasm System.

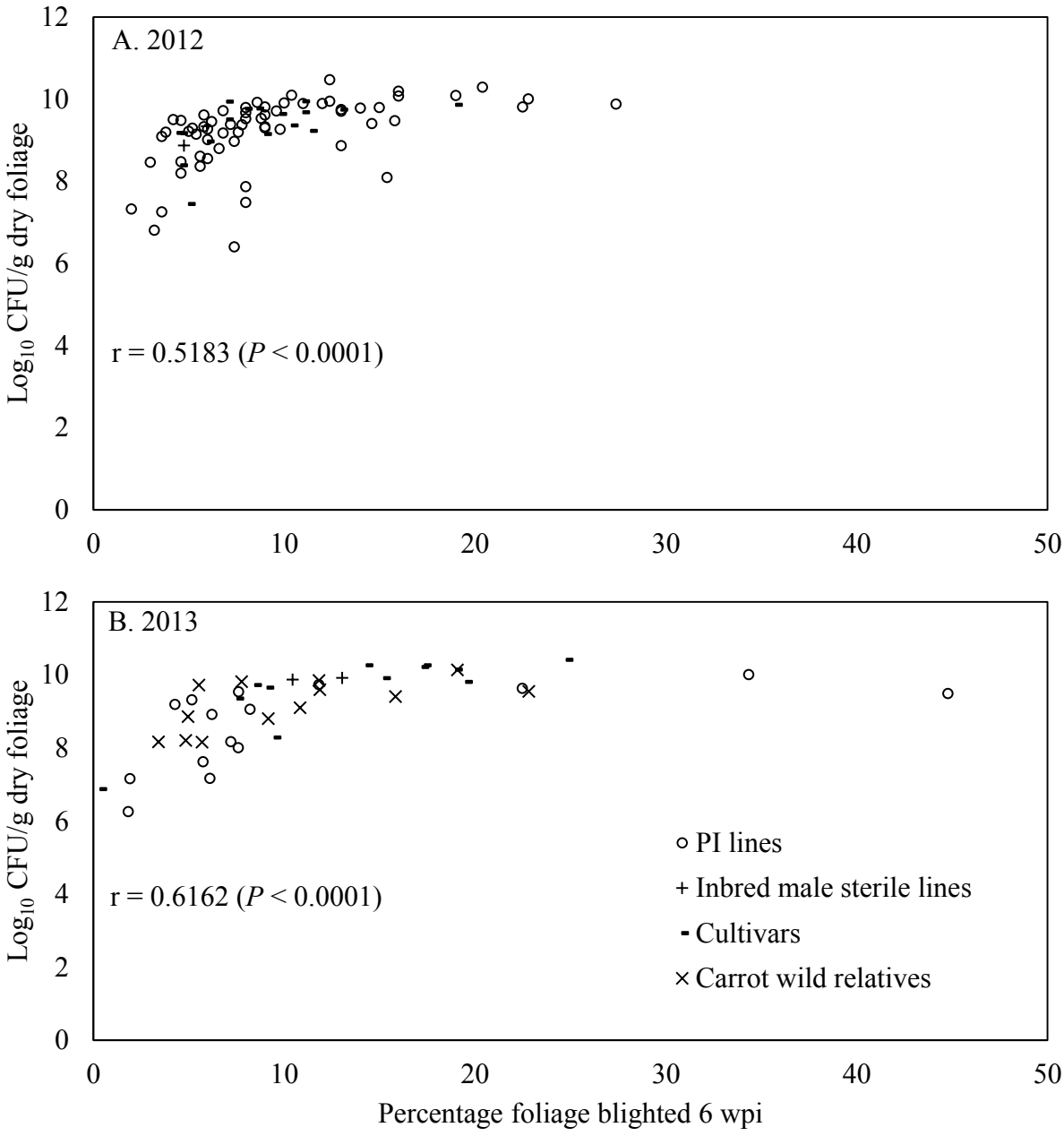

Fig. 2.2. A) *Xanthomonas hortorum* pv. *carotae* population recovered vs. bacterial blight severity observed on carrot foliage measured 6 weeks post inoculation (wpi) from a 2012 greenhouse screening of 66 Plant Introduction (PI) lines, two inbred male-sterile carrot lines (A2566 and A0493), and 17 carrot cultivars. r = Spearman's correlation coefficient (and associated probability, *P*). B) *X. hortorum* pv. *carotae* population vs. bacterial blight severity on carrot foliage measured 6 wpi from a 2013 greenhouse screening of 15 PI lines, the same two inbred male-sterile carrot lines, 8 cultivars, and 13 carrot wild relatives.

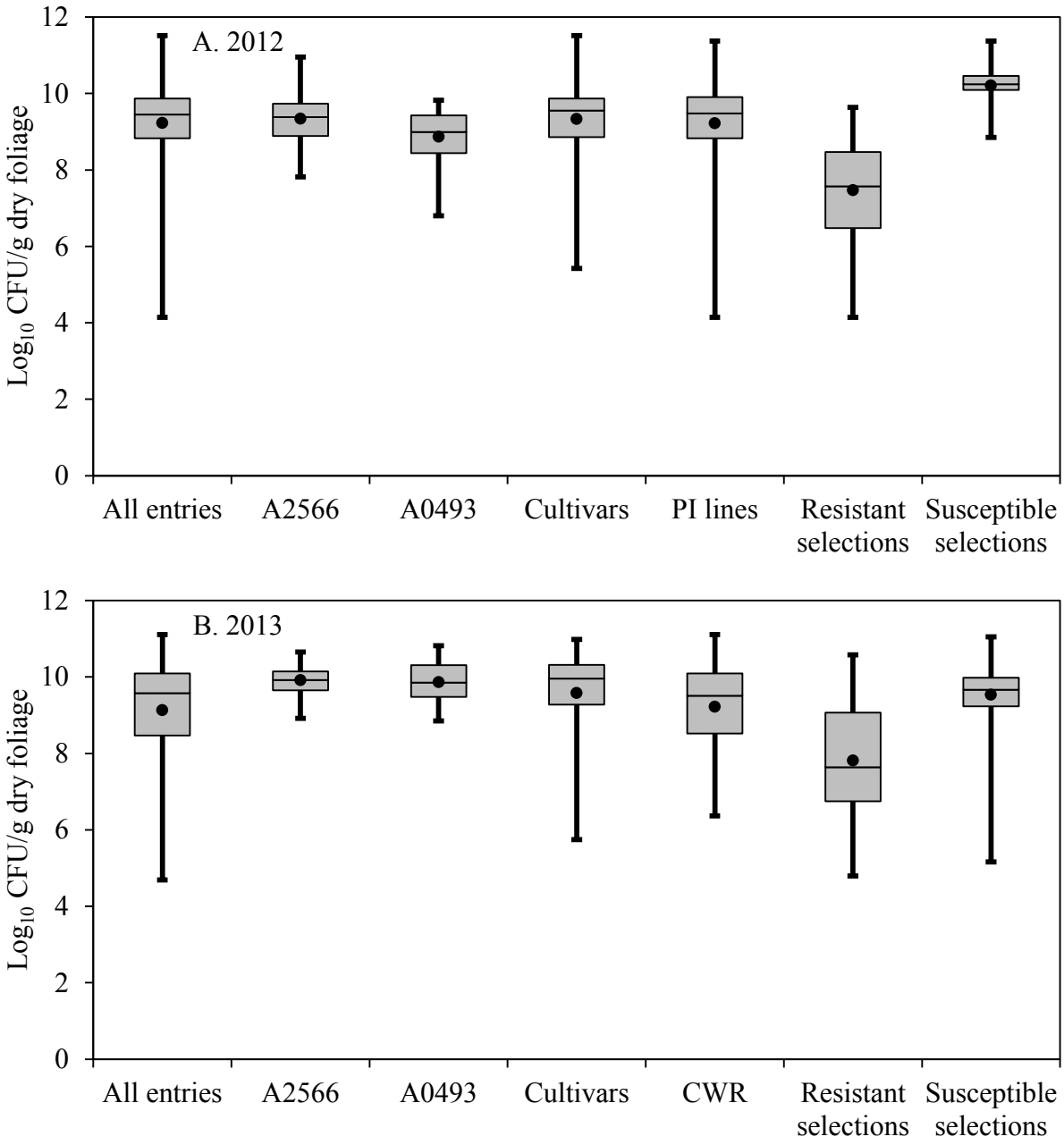

Fig. 2.3. A) Population of *Xanthomonas hortorum* pv. *carotae* detected on the foliage of all 85 carrot lines and cultivars tested, including two inbred male-sterile carrot lines (A2566 and A0493), 17 cultivars, 66 Plant Introduction (PI) lines, 8 resistant selections, and 5 susceptible selections from a 2012 greenhouse screening; and B) all 42 lines and cultivars tested, including the same two inbred male-sterile lines, 8 cultivars, 13 carrot wild relatives (CWR), 8 resistant selections, and 5 susceptible selections tested in a 2013 greenhouse screening. The black dot represents the mean, the horizontal line in each box represents the median, the upper and lower ends of each box represent the first and third quartiles, respectively, and the whiskers represent the range in population of the pathogen detected

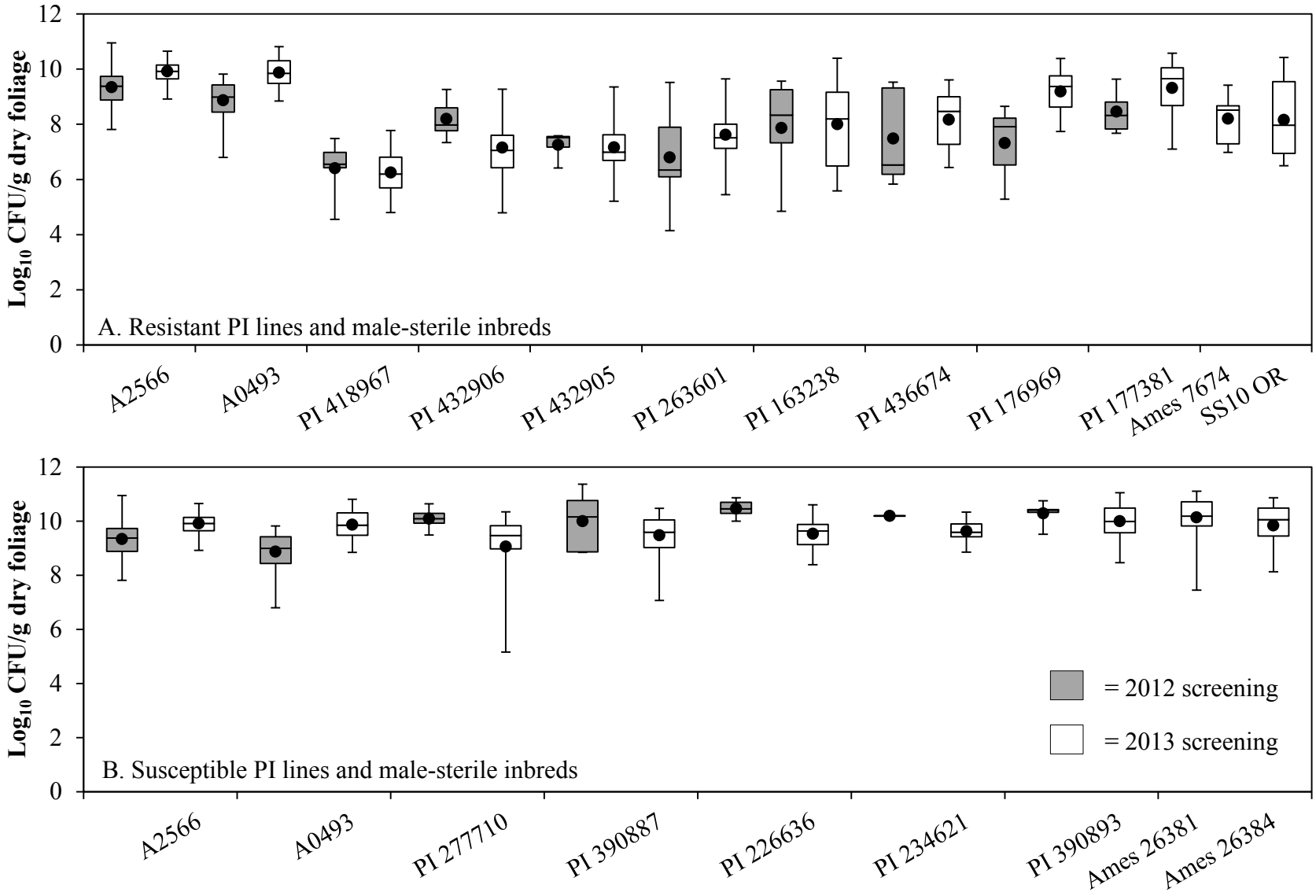
Log10 CFU/g dry foliage
12
10
8
6
4
2
0
A. Resistant PI lines and male-sterile inbreds
A2566
A0493
PI 418967
PI 432906
PI 432905
PI 263601
PI 163238
PI 436674
PI 176969
PI 177381
Ames 7674
SS10 OR
Log10 CFU/g dry foliage
B. Susceptible PI lines and male-sterile inbreds
= 2012 screening
= 2013 screening
A2566
A0493
PI 277710
PI 390887
PI 226636
PI 234621
PI 390893
Ames 26381
Ames 26384

Fig. 2.4. A) Population of *Xanthomonas hortorum* pv. *carotae* detected in 2012 and 2013 greenhouse screenings on the foliage of inbred male-sterile carrot lines (A2566 and A0493), eight carrot Plant Introduction (PI) lines, and two wild relatives (Ames 7674 and SS10 OR) selected as putatively resistant to this pathogen; and B) on the foliage of the same two inbred male-sterile carrot lines as well as five PI lines and two wild relatives (Ames 26381 and 26384) selected as putatively susceptible to this pathogen. The carrot wild relatives were screened only in 2013. The black dot represents the mean, the horizontal line in each box represents the median, the upper and lower ends of each box represent the first and third quartiles, respectively, and the whiskers represent the range in population of the pathogen detected.

Chapter 3

Alternaria spp. associated with seed of *Daucus carota* Plant Introduction lines

3.1. Introduction

Alternaria leaf blight and black rot are caused by *Alternaria dauci* and *A. radicina*, respectively, are economically important diseases in carrot (*Daucus carota*) seed and root production, and have been found in most areas where carrots are produced (Pryor, 2002; Pryor and Strandberg, 2002). *A. dauci* and *A. radicina*, as well as the closely related species *Alternaria carotiincultae* that is also pathogenic on carrot, can be seedborne and seed transmitted, and can cause damping-off of carrot seedlings (Farrar et al., 2004; Pryor, 2002; Pryor and Standberg, 2002).

Alternaria leaf blight is characterized by necrotic leaf lesions, each of which usually is surrounded by a chlorotic halo (Pryor and Strandberg, 2002). Elongated, brown to black lesions can develop on petioles, weakening and eventually collapsing the leaf (Farrar et al., 2004; Pryor and Strandberg, 2002). Alternaria leaf blight primarily causes damage by reducing photosynthetic activity, and can hinder harvest of fresh market carrots if the roots are pulled from the soil by the weakened foliage. Depending on environmental conditions, disease symptoms can appear as soon as 7 to 10 days after infection (Farrar et al., 2004). *A. dauci* has relatively large spores, each with a long beak, which can be distinguished readily from other *Alternaria* spp. associated with carrot. Conidia of *A. dauci* are typically dark brown, 80 to 100 μm x 15 to 24 μm, with the beak of each conidium 200 to 250 μm x 5 μm, and the spores typically do not form in chains (Simmons, 1995; 2007).

Symptoms of black rot typically begin as a black decay at the base of the petioles, resulting in a black ring of rot at the crown of the root (Pryor, 2002). *A. radicina* may also cause foliar lesions similar to Alternaria leaf blight. Additionally, as for Alternaria leaf blight, black rot can impede harvest of fresh market carrots if decayed petioles break while roots are being pulled from the soil by the foliage. Mature conidia are brown, typically 42 to 50 μm x 18 to 25 μm, beakless, and solitary or in clusters of two to three per conidiophore that arise from aerial mycelium or directly from mycelium on the substrate (Simmons, 1995; 2007). Conidia of *A. radicina* are similar in appearance to those of *A. carotiincultae*, a pathogen of carrot that also causes black rot. Pryor and Gilbertson (2002) indicated that *A. carotiincultae* was equally, if not more, virulent on carrot seedlings than *A. radicina*. Conidia of *A. carotiincultae* are, in general, slightly longer than those of *A. radicina,* 40 to 80 μm x 15 to 23 μm (Simmons, 1995; 2007).

An important step in controlling *A. dauci* and *A. radicina* infections in carrot crops is planting pathogen-free seed (Farrar et al., 2004). Seeds infected with *A. radicina* or *A. dauci* can be detected using one of two protocols outlined by the International Seed Testing Association (ISTA, 2013a; 2013b; 2013c; 2013d): 1) a malt agar seed assay, or 2) a freeze blotter seed assay. Infected seed lots can be treated with hot water (50°C for 20 minutes), hot sodium hypochlorite, or fungicides to reduce seedborne infection levels and seed transmission (Pryor, 2002; Pryor et al., 1994; Strandberg, 1984). Fungicide treatments (e.g., azoxystrobin, fludioxonil, iprodione, and thiram) are generally effective for managing seedborne inoculum of *Alternaria* spp. in carrot crops (Pryor, 2002; Strandberg, 1984). However, a low incidence of seed contamination by *A. dauci* (<1%) and *A. radicina* (< 14%) may remain on treated seed lots, which can cause significant infections in root production fields that may be seeded at rates ranging from 0.5 to 3.0 million seeds/ha (Coles and Wicks, 2003; Farrar et al., 2004; Strandberg, 1984).

Foliar fungicide applications (e.g., azoxystrobin, chlorothalonil, iprodione, and pyraclostrobin) may be applied during the growing season to carrot foliage for control of *A. dauci*, and to the crowns or whole carrot roots dipped after harvest and before storage for control of *A. radicina* (Abawi and Ludwig, 2003; Farrar et al., 2004; Lockhart and Delbridge, 1974). Incorporation of carrot crop debris into soil soon after harvest, and crop rotation are also essential for effective management of *A. dauci* and *A. radicina* (Pryor, 2002; Pryor and Strandberg, 2002). Carrot debris that is incorporated into soil decomposes faster than debris left on the soil surface, eliminating most inoculum of *A. dauci* associated with carrot debris post-harvest. However, in contrast to *A. dauci*, Pryor et al. (1998) and Maude and Shuring (1972) found that *A. radicina* could remain viable in soil for as long as four years in California and eight years in the United Kingdom, respectively. Genetic resistance to pathogenic *Alternaria* spp. in carrot is another useful tool to manage Alternaria leaf blight and black rot (Boedo et al., 2009; Farrar et al., 2004; Pryor et al., 2000). Commercial carrot cultivars and inbred lines with different degrees of resistance to Alternaria leaf blight are available (e.g., the partially resistant cultivars Bolero, Carson, B5280, 719116, 713087, and REX-240), although there is only a limited amount of resistance in commercial cultivars to black rot (Pawelec et al., 2006; Pryor et al., 2000; Rogers et al., 2003).

A. dauci belongs to the porri group of *Alternaria* while *A. radicina* belongs to the radicina group (Hong et al., 2005). Other species in the porri group include *A. solani*, *A. brassicae*, and *A. porri*, which are pathogens of plants in the Solanaceae, Brassicaceae, and Alliaceae, respectively (Hong et al., 2005; Pryor and Gilbertson, 2002). The radicina group also includes *A. carotiincultae*, a pathogen of carrot, and *A. petroselini* and *A. smyrni*, pathogens on parsley that have not been reported as pathogenic on carrot (Hong et al., 2005; Pryor and

Gilbertson, 2002). Molecular tools such as DNA fingerprinting using protein-coding gene primers, sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA) or mitochondrial small subunit (mt SSU) rDNA, and random amplified polymorphic DNA (RAPD) analysis have been used successfully to delineate among species in these groups (Hong et al., 2005; Pryor and Gilbertson, 2000; Pryor and Gilbertson, 2002). *A. carotiincultae* and *A. radicina* are nearly indistinguishable based on visual diagnostics alone, but various diagnostic tools (e.g., PCR assays and pathogenicity tests) have been developed to distinguish isolates of the two species (Park et al., 2008; Pryor and Gilbertson, 2002). Among the most effective tools for differentiating between isolates of *A. radicina* and *A. carotiincultae* is the Alt a1 PCR assay (Hong et al., 2005). Additionally, sequences of the Alt a1 gene can be used to separate isolates of *A. dauci* from isolates of closely related members of the porri clade (Hong et al., 2005; Lawrence et al., 2012).

The United States Department of Agriculture (USDA) National Plant Germplasm System (NPGS) has a collection of 1,370 accessions of the genus *Daucus,* including 31 taxa from 64 countries (Reitsma and Clarck, 2013). The USDA NPGS is a valuable source of genetic diversity of various plant species for molecular analyses, adaptation studies, morphological and germination studies, and genetic and breeding efforts. For example, in 2009 and 2010, carrot seed from the USDA NPGS was distributed throughout the United States to 39 requestors, and to five requestors from other countries (USDA NPGS Root and Bulb Vegetable Crop Germplasm Committee, 2010). However, distribution of seed also represents a potential for spread of inoculum of any seedborne pathogens that might be associated with the seed. Villarroel-Zaballos et al. (2012) showed that some seed of spinach Plant Introductions obtained from the USDA NPGS was infested with pathogenic *Verticillium dahliae,* and that the infested seed caused

Verticillium wilt to develop on non-inoculated plants in a *V. dahliae* resistance screening study. In this carrot project, damping-off symptoms were observed on seedlings of carrot accessions from the USDA NPGS during the 2012 *Xanthomonas hortorum* pv. *carotae* carrot resistance screening (see Chapter 2). When isolations were carried out from the symptomatic carrot seedlings, conidia resembling those of *A. dauci* and *A. radicina* were observed. Given the seeborne nature of *A. dauci* and *A. radicina*, the potential economic importance of these pathogens, and the global distribution of *Daucus* spp. germplasm from the USDA NPGS, the objective of this study was to identify *Alternaria* spp. associated with seed of a subset of the *Daucus* germplasm collection from the USDA NPGS that was screened for resistance to *X. hortorum* pv. *carotae* (see Chapter 2).

3.2. Materials and Methods

Seed health assay. Seed from 66 carrot Plant Introduction (PI) lines were obtained from the USDA NPGS as described in Chapter 2. Damping-off symptoms were observed when seed of some of the PI lines were planted in a greenhouse for the 2012 *X. hortorum* pv. *carotae* resistance screening (see Chapter 2). The potting medium used in the 2012 *X. hortorum* pv. *carotae* resistance screening was pasteurized. Therefore, the damping-off could not have been caused by soilborne pathogens in the potting medium. Symptomatic hypocotyl and root sections from the seedlings were cut into approximately 5 mm long pieces; surface-sterilized in 1% sodium hypochlorite for 10 to 30 s; triple-rinsed in sterilized, deionized water; dried on sterilized blotter paper; and placed, partially submerged, onto potato dextrose agar (Difco Laboratories, 1953) amended with 100 ppm chloramphenicol (cPDA), as well as water agar [15g Bacto agar (Becton Dickinson, Franklin Lakes, NJ) per 1 liter water] amended with 100 ppm

chloramphenicol (cWA). Fungal growth from the carrot sections was monitored for 3 days. Conidia resembling those of *A. dauci, A. radicina*, and *A. carotiincultae* were observed on the tissue pieces.

Seed of each of the 66 carrot PI lines was assayed using the malt agar protocol for detection of *A. dauci* and *A. radicina*, as described by the International Seed Testing Association (ISTA, 2013b; 2013d). Malt agar was poured into 11.0 cm x 11.0 cm x 2.9 cm, sterilized, 91C transparent, rectangular boxes, each with a friction-fitting lid (Hoffman Manufacturing, Inc., Albany, OR). Twenty-five seeds from each of the 66 PI lines were placed, evenly spaced and partly submerged, onto the agar medium in one container. The seed were then incubated in the boxes at 20 ± 2°C with 12 h/12 h night/day cycles, and both near-UV and cool-white fluorescent light by day. After five days of incubation, each seed was examined microscopically (x40 to x100) for conidia of *A. dauci* and *A. radicina*. The number of seeds infested with *A. dauci* and/or *A. radicina* was recorded for each PI line (Table 3.1).

Individual spores of suspected *A. dauci* and *A. radicina* were removed from infested seeds with a sterilized needle, placed on a plate of cPDA, and incubated at room temperature. Each single-spore isolate was subcultured similarly two or three times to ensure isolate purity. Isolates of suspected *A. dauci* (n = 17) and *A. radicina* (n = 27) from the seed of 23 PI lines were procured in this manner. After sub-culturing, the isolates were each grown at approximately 20°C on a plate of cPDA on which four or five 413 grade, 1.5 cm-diameter filter discs (VWR International, LLC, Radnor, PA) were placed. When the filter discs were fully colonized by mycelium of an isolate, the discs were transferred to a sterilized, 5.1 cm x 5.1 cm coin envelope using sterile technique, and the coin envelope and filter disks were placed in a laminar flow hood overnight to dry. The filter discs in the coin envelopes were then stored in 7.5 cm x 7.5 cm x 7.5

cm, GA-7 tissue culture vessels (Magenta LLC, Chicago, IL) with Drierite desiccant (W. A. Hammond Drierite Co., Xenia, OH) at -20°C for long-term storage.

Identification of *Alternaria* spp. *Morphological identification.* The isolates of *Alternaria* spp. obtained from seed of carrot PI lines were grown on cPDA and incubated at 20 ± 2°C on a lab bench for ≥ 10 days. Conidia from each isolate were examined with a microscope (x8 to x100). Isolates with single conidia that did not form chains, and that each had a beak approximately 100 to 200 μm long were identified as putative *A. dauci* isolates. Additionally, all putative *A. dauci* isolates produced a red to brown pigment on cPDA, which is characteristic for *A. dauci* (Freeman, 1965). Isolates with multiple conidia per conidiophore, no chains of conidia, no beaks on the conidia, and conidiophores arising from aerial mycelia were labeled as putative *A. radicina* or *A. carotiincultae* isolates. *A. radicina* isolates typically produce a yellow pigment on cPDA after ≥10 days of growth, whereas *A. carotiincultae* isolates do not produce any pigment on cPDA (Pryor and Gilbertson, 2002).

***DNA extraction protocols*.** Approximately 100 mg mycelium of each isolate growing on a cPDA plate was scraped off the plate with a sterilized metal weighing spoon, and placed in a 1.5 ml microcentrifuge tube with 0.5 ml of 0.5 mm-diameter glass beads (Biospec Products, Inc., Bartlesville, OK). The tubes were each then placed at -80°C for ≥ 12 h. The frozen tubes were then placed in a Mini-Beadbeater (Biospec Products, Inc.) operated for 40 s at 36 oscillations/s, frozen again at -80°C for 30 min, and returned to the beadbeater for disruption for another 40 s at 36 oscillations/s. DNA from each putative *A. dauci* isolate was extracted from the pulverized mycelium using the DNeasy Plant Minikit (Qiagen Inc., Valencia, CA). DNA of each putative *A. carotiincultae* isolate was extracted using the FastDNA kit (MP Biomedicals, LLC, Santa Ana, CA) because high quality DNA could not be attained readily from these isolates using the

DNeasy Plant Minikit (*data not shown*). DNA of each isolate was quantified using the Qubit fluorometer (Life Technologies, Carlsbad, CA).

DNA of each putative *A. radicina* isolate was extracted using a protocol based on that of Dobinson (1995) and Peever et al. (1999) because PCR assays did not yield amplicons of the expected length (~510 bp) when using DNA extracted with the DNeasy Plant Minikit or the FastDNA kit (*data not shown*). Therefore, for each putative *A. radicina* isolate, 1 ml sterilized water was pipetted onto each agar plate colonized by the isolate, and conidia dislodged using a sterilized, bent glass rod. Approximately 750 µl of the spore suspension was then pipetted into 100 ml of 2-YEG broth (Stewart et al., 2013) in 250 ml Erlenmeyer flasks. Each flask was placed on a rotary shaker operated at 120 rpm for 4 days (Pryor and Gilbertson, 2000). Mycelium was harvested from each flask, vacuum-filtered through Whatman no. 1 qualitative filter discs (70 mm diameter, GE Healthcare Corp., Little Chalfont, Buckinghamshire, UK), frozen at -80°C for 24 h, lyophilized, and stored at -20°C.

Approximately 100 mg of lyophilized mycelium of each isolate was placed in a non-conical, 2 ml microcentrifuge tube, and crushed using a sterilized metal weighing spoon. One ceramic bead (0.64 cm-diameter ceramic sphere, MP Biomedicals, LLC) was placed in each microfuge tube with the crushed mycelium, and vortexed vigorously to pulverize the mycelium further. A 400 µL aliquot of lysis buffer (0.2 M Tris HCl, pH 8.0; 0.5 M NaCl; 0.01 M EDTA; 1% SDS; and water added to a final volume of 125 ml) was added to the pulverized mycelium, and the tubes alternated between 30 s vortex and 30 s on ice for 3 min (Dobinson, 1995). Phenol chloroform was added to the sample (450 ul at 1:1) and the sample vortexed for 30 s. The supernatant was separated by centrifugation (10,000 rpm) for 5 min, and re-extracted with phenol chloroform. Each DNA sample was then precipitated twice with 95% ethanol, stored on

ice for 3 min, and centrifuged (10,000 rpm) for 5 min. The nucleic acid pellet was then washed with 70% ethanol and centrifuged (16,000 rpm) for 5 min. The ethanol was drained from each tube, taking care not to dislodge the nucleic acid pellet. Each tube was placed in a vacufuge and spun for 30 min at 30°C under vacuum to evaporate the ethanol completely. The pellet was re-suspended in 100 µl of 1X TE buffer by manual vortexing. After the DNA was re-suspended, 2 µl of 1 mg/ml RNAse was added to the DNA suspension, and the tube incubated for 3 h at 37°C to digest any RNA. The extracted DNA was visualized in 2% stained (SYBR Safe DNA gel stain, Life Technologies) agarose gels, and the amount of DNA quantified by comparing the brightness of genomic DNA bands visualized with 15, 31, 63, and 125 ng DNA standards (Lambda DNA, Life Technologies).

Alt a1 PCR assays and sequencing. DNA extracts were then used in PCR assays with the Alt a1 forward primer (5'-ATGCAGTTCACCACCATCGC-3') and reverse primer (5'-ACGAGGGTGAYGTAGGCGTC-3') designed by Hong et al. (2005). The PCR mixture contained 1x PCR buffer II (Applied Biosystems, Foster City, CA), 2.5 mM $MgSO_4$, 10 ng template genomic DNA, 2 pmol of each primer, 200 µM dNTPs (Qiagen, Inc.), and 1 U of AmpliTaq polymerase (Applied Biosystems), with the total volume adjusted to 25 µl using deionized water. The PCR assay for each *A. dauci* isolate was conducted using an initial denaturation at 94°C for 5 min; followed by 35 cycles of 94°C for 40 s of denaturing, 58°C for 40 s of annealing, and 72°C for 1 min of extension; and a final extension step of 72°C for 15 min. PCR assays for the putative *A. radicina* and *A. carotiincultae* isolates entailed the same conditions except the annealing temperature started at 61°C and decreased 0.1°C for each of 20 cycles to reach a final annealing temperature of 59°C for the last 15 cycles. For all isolates, the amplified products were visualized in 2% SYBR Safe-stained agarose gels. A 10 µL aliquot of

each PCR product was mixed with 1 µL of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30.00% glycerol, and 50 mM EDTA), 10 µL of the mix loaded into each agarose gel well (one mix/well), and the gel electrophoresed for 1.5 h at approximately 96 volts to separate amplified DNA products. The gel was then visualized with ultraviolet light using AlphaImager software (ProteinSimple, Inc., Santa Clara, CA).

When multiple fragment lengths were observed for a PCR assay of an isolate, the target size fragment was excised from the 0.5% agarose gel, and the PCR amplicon extracted using a Qiaex II Gel Extraction Kit (Qiagen, Inc.). Extra nucleotides, primers, and other contaminants in the PCR product mix that may hinder sequencing of PCR products were digested using ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA). The amplified, cleaned product was then quantified using a Qubit fluorometer. The forward and reverse sequences of each PCR product were then determined by ELIM Biopharmaceuticals, Inc. (Hayward, CA). Forward and reverse sequences were proofread, aligned manually, and edited in BioEdit 7.2.5 (Ibis Biosciences, Carlsbad, CA). The consensus sequence for each isolate was then submitted to GenBank (Table 3.2).

Carrot root disc pathogenicity test. In fall 2013, 27 isolates of *A. radicina*, 2 isolates of *A. carotiincultae*, and 1 isolate of *A. petroselini* were tested for pathogenicity on carrot root discs to assess if the isolates can cause black rot symptoms. One isolate of *A. dauci* was also tested as a negative control treatment. The isolates were grown on half-strength cPDA (19.5 g PDA, 7.5 g agar, and 1 liter water, amended with 100 ppm chloramphenicol). Of the 27 *A. radicina* isolates, 25 were from carrot seed of PIs from the USDA NPGS and 2 were from Dr. Barry Pryor at the University of Arizona. The *A. dauci* isolate was from a carrot seed of PI 390887. Of the two *A. carotiincultae* isolates, one was obtained from carrot seed of PI 418967 and one was from Dr. Barry Pryor, and the *A. petroselini* isolate was from PI 280706 (Tables 3.2 to 3.3). Each isolate

was grown for at least 1 week until the mycelium had covered most of the surface of the plate. Based on the method described by Pryor et al. (1994), healthy, mature carrot roots from a local market in Skagit Co., WA were washed thoroughly under tap water. Two Whatman No. 1 filter paper discs (90 mm diameter) were placed in a 100 mm-diameter glass Petri dish, onto which 2 ml of a 0.05% streptomycin sulfate + 0.05% chlortetracycline HCl antibiotic solution were pipetted. Carrot disks (each approximately 0.5 to 0.75 cm thick) were cut from the roots, surface-sterilized in 1% sodium hypochlorite for 5 min, and triple-rinsed in sterilized, deionized water. Sterilized carrot discs were then placed on the filter paper in the Petri dishes (one carrot disc/Petri dish). A colonized agar plug cut from the margin of an actively growing isolate was placed onto the carrot disc with the colonized surface facing the disc. The agar plug and carrot disc were then moistened with the antibiotic solution. Three replicate carrot discs per isolate were set up in a completely randomized design. The plates with the root discs were placed randomly on wire racks in a 14.2 liter, plastic latch box (1884 model, Sterilite Corp., Townsend, MA) with approximately 100 ml water added to the bottom of each box to maintain high relative humidity. A lid was placed over each box, and the discs incubated for 10 days at room temperature (22 ± 1°C).

After 10 days of incubation, each Petri dish was removed from the box, and pathogenicity rating of the isolate completed using a 0-to-9 scale, where: 0 = no discoloration of the disc; 1 = slight discoloration; 2 = softening and necrosis of the disc; 3 = disc rotted slightly on the surface and edges, and on which sporulation typical of *A. radicina* was observed microscopically; 6 = disc rotted moderately with part of the disc succumbing to soft rot, and conidia typical of *A. radicina* observed on the disc; and 9 = disc rotted entirely with conidia typical of *A. radicina* observed microscopically. This rating scale was adjusted from that described by Pryor et al.

(1994), because severity of the carrot disc rot varied substantially among isolates, but the scale described by Pryor et al. (1994) did not account for severity of disc rot, only severity of discoloration.

Carrot foliar pathogenicity test. In spring 2014, seven seeds of the carrot cultivar Big Sur (Nunhems USA, Inc.; Parma, ID) were sown in Sunshine Mix #1 in 12.7 cm-diameter pots (STD, Anderson Die and Manufacturing Inc., Portland, OR) in a greenhouse, with 7 seeds/pot. Plants were fertigated as needed (Wil-Sol Pro-Balance 20-20-20 Superior Water Soluble Professional Turf and Ornamental Fertilizer, Wilbur-Ellis, San Francisco, CA; injected at 200 ppm N). Insect pests were controlled with a weekly spray of acephate (Orthene Turf, Tree, and Ornamental Spray 97, Amvac Chemical Corp., Los Angeles, CA), spinosad (Entrust Naturalyte Insect Control, Dow AgroScience LLC, Indianapolis, IN), imidacloprid (Leverage 2.7, Bayer CropScience LP, Research Triangle Park, NC), or *Beauvaria bassiana* (Botanigard 22WP, Laverlam International Corp., Butte, MT). After appearance of the first true leaf, carrot plants were thinned to five/pot. Thirty-five days after planting, the pots were placed in a plastic bin (four pots/bin), and each bin enclosed in a 38.0 cm x 22.9 cm x 81.3 cm plastic bag (U.S. Plastic Corp., Lima, OH) for 24 h prior to inoculation to increase relative humidity and promote opening of stomata on the carrot leaves.

Three isolates of *A. dauci,* five isolates of *A. radicina,* three isolates of *A. carotiincultae,* and one isolate of *A. petroselini,* identified based on sequencing of the Alt a1 gene region or in a previous study (Park et al., 2008), were grown on half-strength cPDA for ≥10 days (Table 3.4). On the day of inoculating carrot plants, each plate of each *Alternaria* isolate was flooded with 40 ml 0.01% Tween 80 (Sigma Aldrich Corp., St. Louis, MO). Conidia on each plate were dislodged with a sterilized, bent glass rod, and each spore suspension was filtered through two

layers of cheesecloth to remove hyphal fragments. The concentration of each spore suspension was determined using a hemocytometer, and diluted to 2.0 x 10^3 conidia/ml. However, the suspension of *A. dauci* isolate Adc013 could only be concentrated to 1.1 x 10^3 conidia/ml because of limited spore production.

The carrot plants were removed from the plastic bags, and the foliage of plants in four replicate pots sprayed with the conidial suspension of the appropriate isolate until runoff (approximately 3 ml/five plants/replicate pot) using an airbrush sprayer (Model 175, Badger Air-Brush Co., Franklin Park, IL). Non-inoculated control plants in four replicate pots were sprayed similarly with 0.01% Tween 80. Inoculated and control carrot plants were then placed back in the same plastic bins (four pots/bin), and each bin enclosed in a plastic bag for 48 h. The pots of carrots were then removed from the bags and bins, and placed in a greenhouse at 25 ± 1°C in a randomized complete block design with four replicate blocks. The carrot foliage was monitored for symptoms 1, 2, 3, and 4 weeks post-inoculation (wpi). Disease symptoms were rated based on the percentage foliage with lesions. Four wpi, isolations were carried out from symptomatic leaf tissue of carrot plants inoculated with each isolate. Symptomatic foliar tissue was surface-sterilized in 1% sodium hypochlorite for 30 s, and placed on cPDA in 100 mm-diameter Petri dishes. The plates were stored at 20 ± 2°C for 5 days under ambient light in a laboratory next to a north-facing window, and the foliar pieces were examined microscopically for conidia of *Alternaria* spp.

Parsley foliar pathogenicity test. A parsley foliar pathogenicity test was also conducted because one putative isolate of *A. petroselini*, a pathogen of parsley but not of carrots, was obtained from a seed of the carrot PI 280706, with the species identified based on the Alt a1 PCR assay and consensus sequence comparison with the NCBI database (see Results). Seeds of the

parsley cultivar Titan (Bejo Seeds Inc., Oceano, CA) were sown in pots, and the plants thinned and managed as described for the carrot foliar pathogenicity test. Thirty-seven days after planting, the pots were placed in plastic bins (two pots/bin) and each bin enclosed in a plastic bag for 24 h.

A conidial suspension of each of three *A. petroselini* isolates as well as one *A. dauci* isolate, one *A. radicina* isolate, and one *A. carotiincultae* isolate were prepared as described for the carrot foliar pathogenicity test (Table 3.4). The conidial suspension of *A. dauci* isolate Adc041 was filtered through one layer of cheesecloth because of the minimal number of spores recovered for this isolate using two layers of cheesecloth. The spore suspension of each isolate was used to inoculate plants in two replicate pots using the method described for the carrot foliar pathogenicity test. Non-inoculated control plants in two replicate pots were sprayed similarly with 0.01% Tween 80. Pots were then placed in a randomized complete block design on a greenhouse bench, with two replicate blocks. Foliar disease ratings and isolations from parsley leaves were completed as described for the carrot foliar pathogenicity test.

Data analysis. Data were analyzed using SAS Version 9.3 (SAS Institute, Cary, NC). The Kruskal-Wallis analysis of variance (ANOVA) was run on the ordinal carrot disc rot ratings from the carrot root disc pathogenicity test using PROC NPAR1WAY. The full data set for all weekly ratings for both the carrot and parsley foliar pathogenicity tests did not meet the assumptions of normally distributed residuals and homogeneity of variance required for parametric analysis, and no transformation satisfied these assumptions. Therefore, repeated measures analysis was conducted on ranked percentage foliar blighting for both the carrot and parsley pathogenicity tests using PROC MIXED to compare the percentage foliar blighting among sample times (1, 2, 3, and 4 wpi). The compound symmetry covariance structure was

used for repeated measures analysis because this had the best fit. For the 4 wpi ratings, log transformation of the carrot foliar pathogenicity test data was used as this met assumptions for parametric analysis. Similarly, rank transformation of the parsley foliar pathogenicity test data was performed because other transformations of the data did not meet assumptions for parametric analysis. Therefore, means comparisons using Fisher's protected least significant difference (LSD at $P < 0.05$) were carried out on log-transformed disease ratings 4 wpi and ranked disease ratings 4 wpi for both the carrot and parsley foliar pathogenicity tests, respectively, using PROC MIXED.

3.3. Results

Seed health assay. Mycelial growth was observed on seed of all 66 carrot PI lines plated in the malt agar seed health test (Fig. 3.1). However, most of the spores observed microscopically resembled those of *A. alternata* with numerous, relatively small conidia (approximately 30 μm x 12 μm) present in chains. Out of 25 seeds tested/PI, the average number of seeds infested with putative *A. radicina* and *A. dauci* was 1.9 and 3.1 among all 66 PI lines, respectively. Of the 66 PI lines tested, putative *A. radicina* conidia were detected on seed of 37 PIs at a range of 4 to 80% infested seed (mean of 14%), putative *A. dauci* conidia were detected on seed of 34 PIs at a range of 4 to 100% (mean of 24%), and both putative *A. radicina* and *A. dauci* conidia were detected on seed of 21 PIs. Seed lots of only 16 PI lines had no putative *A. radicina* or *A. dauci* conidia observed (Table 3.1). Of the 34 PIs on which *A. dauci* was observed on the seed, 5 PIs had >50% of the seed infested with *A. dauci*, while just one PI had >50% seed infested with *A. radicina* of the 37 infested with that species (Table 3.1). PI 251228 had the greatest incidence of seed infested with *A. radicina* (80%) (Table 3.1). PI

251228 also had 16% of the seed infested with *A. dauci*. PI 169488 had the greatest incidence of seed infested with *A. dauci* (100%), and 28% of the seed was also infested with *A. radicina* (Table 3.1). A total of 27 putative *A. radicina* isolates were obtained from seed of 17 PI lines, and 17 putative *A. dauci* isolates obtained from seed of 12 PI lines. The identities of these isolates were confirmed in subsequent morphological, molecular, and pathogenicity studies as described below.

Identification of *Alternaria* spp. Colonies of putative *A. radicina* isolates growing on cPDA had irregular margins, grew relatively slowly (average 2 cm-diameter colonies after 10 days on cPDA), and developed conidia on conidiophores that formed on aerial mycelium as well as directly on the agar medium, with the conidia present individually or in groups of two to three (Fig. 3.2B). After 10 days of growth, all but two putative *A. radicina* isolates produced a yellow pigment on cPDA. The two isolates, Acn010 and Apt007, were subsequently identified as one isolate each of *A. carotiincultae* and *A. petroselini* by sequencing the Alt a1 gene and comparing the sequences with Alt a1 sequences in GenBank (see below). Conidia examined microscopically of putative *A. radicina* isolates were beakless, ellipsoid or ovoid, and ranged from 20 to 50 μm x 15 to 20 μm (Fig. 3.3D). Colonies of putative *A. dauci* isolates had regular margins and grew relatively rapidly (average 9 cm-diameter colonies after 10 days on cPDA). Conidia of putative *A. dauci* isolates were solitary on conidiophores that developed directly from the agar medium and, occasionally, on aerial mycelia (Fig. 3.2D). The conidia had beaks that were 100 to 200 μm x 5 μm, and the spores were obclavate and ranged from 40 to 80 μm x 15 to 25 μm (Fig. 3.3B). All putative *A. dauci* isolates produced a red to brown pigment on cPDA after 10 days.

Alt a1 PCR assays for 36 *Alternaria* isolates suspected of being pathogenic on carrot produced an amplicon of ~510 bp for each isolate. A BLAST search of the GenBank database with the consensus sequence of each isolate revealed the Alt a1 gene of Apt007 had 100% sequence identity with the Alt a1 sequence of *A. petroselini* Accession AY563288 (isolate BMP 0144) (Table 3.2). The Alt a1 gene of Acn010 had 100% sequence identity with that of *A. carotiincultae* Alt a1 Accession EU139320 (isolate BMP 0133) (Table 3.2). The Alt a1 gene sequences of 16 putative isolates of *A. radicina* had 100% sequence identity with the Alt a1 sequence of *A. radicina* isolate BMP 0098 (EU139335); and the Alt a1 sequences of two additional isolates, Ard047 and Ard039, had 99% sequence identity with that of the reference isolate, BMP 0098 (Table 3.2). The Alt a1 sequences of all 16 putative isolates of *A. dauci* had 100% sequence identity with the Alt a1 sequence of *A. dauci* isolate BMP 0167 (HE796725) (Table 3.2). The Alt a1 sequences for each of seven putative *A. radicina* isolates (Ard021, Ard025, Ard042, Ard046, Ard049, Ard053, and Ard058) could not be obtained since the PCR assay did not amplify the target sequence at a concentration at which gel extraction or sequencing of the PCR product could be carried out successfully.

Carrot root disc pathogenicity test. All *A. radicina* isolates collected from carrot seed caused rotting of carrot discs using the protocol of Pryor et al. (1994). Carrot disc rot ratings ranged from 1 to 9 and averaged 5.4 ± 0.2 among all *A. radicina* isolates and one *A. dauci* isolate tested. However, the Kruskal-Wallis test of ranked carrot disc rot ratings showed no significant effect of isolate on severity of rot with the experimental design used in this study ($P = 0.0846$). Isolate Ard027 caused the most severe rot of carrot discs (mean of 8) but this was not significantly different compared to that of other isolates (Table 3.3). *A. dauci* isolate Adc038

caused an average carrot disc rot of 1 (Table 3.3). Non-inoculated control discs did not display any rot.

Carrot foliar pathogenicity test. All five *A. radicina* isolates, three *A. carotiincultae* isolates, and three *A. dauci* isolates caused blight symptoms on carrot foliage, but the *A. petroselini* isolate (Apt007) was not pathogenic (Fig. 3.4 and Table 3.4). Repeated measures analysis using the compound symmetry covariance structure on ranked percentage carrot foliage blighted showed a significant effect ($P < 0.0001$) of isolates of *Alternaria* spp. on blight symptoms over the 4-week period following inoculation. In addition, there was a significant effect of weekly rating ($P < 0.0001$) but no significant interaction between isolates and weekly ratings ($P = 0.6860$). Ranked foliar blight ratings at week 4 (2.5 ± 0.4%) were significantly more severe than ratings for earlier weeks; ratings at weeks 2 and 3 (1.4 ± 0.2 and 1.6 ± 0.3%, respectively) were not significantly different, but were significantly more severe than week 1 ratings (0.4 ± 0.0%). *A. radicina* isolate BMP 0047 caused the most severe blight on carrot foliage 4 wpi with an average 5.25% blighting, but this was similar statistically to severity of blight caused by eight other isolates. Only Adc013 and BMP3127, two *A. dauci* isolates that each caused 1.00% blighting, caused less severe blighting than BMP 0047 (Table 3.4). *A. petroselini* isolate Apt007 did not cause any symptoms on carrot foliage 4 wpi (Table 3.4). Non-inoculated control plants had an average 0.25% foliage blighted.

Isolations from symptomatic carrot foliage produced conidia of *A. carotiincultae* on carrot foliar pieces that had been inoculated with isolates Acn010, BMP 0132, and BMP 0134. *A. dauci* conidia developed on carrot foliage inoculated with isolates Adc013, Adc041, and BMP 3127. Conidia of *A. radicina* were observed on carrot foliar pieces inoculated with isolates

Ard038, Ard039, Ard049, and Ard059. Isolations from foliage of control plants did not produce any fungal growth.

Parsley foliar pathogenicity test. All three *A. petroselini* isolates, the *A. radicina* isolate, and the *A. dauci* isolate caused blight symptoms on parsley foliage (Fig. 3.5 and Table 3.4). Repeated measures analysis on ranked percentage parsley foliage blighted showed that isolates of *Alternaria* spp. had a significant effect ($P < 0.0001$) on severity of blight symptoms over the 4 week period following inoculation, weekly ratings differed significantly ($P < 0.0001$), and there was a significant interaction of isolates with weekly ratings ($P = 0.0258$). Ranked foliar blight ratings for weeks 1 and 2 (0.2 ± 0.1% for both weeks) were significantly less than ratings for weeks 3 and 4 (1.1 ± 0.5% and 1.5 ± 0.6%, respectively), with no significant difference between the latter 2 weeks. *A. petroselini* isolate Apt007 caused the most severe disease on parsley foliage 4 wpi with an average 5.00% blighting, which was similar statistically to severity of blighting caused by *A. petroselini* isolate BMP 0144 (3.00%) (Table 3.4). *A. carotiincultae* isolate Acn010 did not cause symptoms on parsley foliage, and *A. dauci* isolate Adc041 as well as *A. radicina* Ard049 caused only 0.25% blighting, which was similar to severity of symptoms observed on non-inoculated control plants (Table 3.4). However, symptoms observed on non-inoculated control plants were likely caused by abiotic stress since isolations from these plants did not produce any fungal growth. Isolations from symptomatic parsley leaf tissue resulted in development of conidia of *A. petroselini* on parsley foliage that had been inoculated with isolates Apt007, BMP 0142, and BMP 0144. Conidia of *A. dauci* and *A. radicina* were observed on parsley foliage inoculated with isolates Adc041 and Ard049, respectively.

3.4. Discussion

To our knowledge, this is the first report of *A. carotiincultae*, *A. dauci*, and *A. radicina* detected on the seed of PI lines from the USDA NPGS, and of *A. petroselini* isolated from carrot seed in general. *A. radicina* and *A. dauci* isolates were found on the seed of 56 and 52% of the carrot PIs tested, respectively, and 32% of the PIs tested had both *A. radicina* and *A. dauci* conidia present on the seed. All but one (Apt007) of the 36 isolates were confirmed as pathogenic species of carrot by sequencing the Alt a1 gene, although isolates were not generated of *Alternaria* spp. that were typical of species not pathogenic on carrot, e.g., *A. alternata*. A majority of the 36 isolates were *A. dauci* (16 isolates) or *A. radicina* (18 isolates), two of the most common pathogens of carrot (Farrar et al., 2004). Only one isolate of *A. carotiincultae* (Acn010), a pathogen that causes similar symptoms on carrot plants as those caused by *A. radicina* but that typically is detected less frequently on carrot (Farrar et al., 2004; Pryor and Gilbertson, 2002), was isolated from one seed of PI 418967. Only 25 seeds of each the carrot PIs were assayed using the malt agar carrot seed assay in this study because of the limited number of seeds distributed by the USDA NPGS (approximately 200 seeds/PI), most of which were used in the 2012 and 2013 *X. hortorum* pv. *carotae* resistance screening trials (see Chapter 2).

The mycelia of *A. carotiincultae*, *A. petroselini*, and *A. radicina* isolates colonized and caused mild to severe rot of carrot discs in the carrot root disc pathogenicity test. Although there was much variation in carrot root disc severity ratings in this test, the variation in severity of rot was not significant among isolates ($P = 0.0846$). Pryor et al. (1994) verified that *A. radicina* isolates caused significantly more rot on carrot roots than *A. dauci* isolates. However, *A. carotiincultae* and *A. petroselini* isolates were not tested by Pryor et al. (1994). Although the

carrot root disc pathogenicity test showed no significant difference among isolates at causing rot of root discs, the results are similar to those of Pryor et al. (1994) in that the *A. dauci* isolate averaged a carrot root disc rot severity rating of 1, less than that of all the *A. radicina* isolates tested, (average ≥ 3). Additionally, unlike in the study of Pryor et al. (1994), carrot root disc rot ratings were on an ordinal scale of 1 to 9, rather than 1 to 3, to assess smaller degrees of difference in the severity of rot. A more robust pathogenicity test, i.e., with a greater number replications of treatments, may have detected significant differences among the *Alternaria* isolates in ability to cause black rot, as only three replications were utilized in this study, and the trial was not repeated.

Despite the relatively limited number of isolates of *A. carotiincultae* (n = 3), *A. dauci* (n = 3), and *A. radicina* (n = 5) evaluated in the carrot foliar pathogenicity test, all of the isolates were pathogenic on carrot. The *A. petroselini* isolate was not pathogenic on carrot but was pathogenic on parsley foliage. Isolates of *A. dauci* and *A. radicina* also caused foliar blight symptoms on parsley foliage. However, the symptoms caused by *A. dauci* and *A. radicina* generally were less severe, with two of the three *A. petroselini* isolates causing significantly more severe blighting on parsley foliage than isolates of the former two species. These foliar pathogenicity tests were designed based on protocols used by Pryor and Gilbertson (2002), but the pathogenicity test on each host was not conducted in this study. Similar to results of the carrot and parsley foliar pathogenicity tests by Pryor and Gilbertson (2002), *A. petroselini* was significantly more virulent on parsley foliage than *A. carotiincultae* and *A. radicina*, with no significant difference between the latter two species; and *A. carotiincultae* and *A. radicina* were significantly more virulent on carrot foliage than *A. petroselini*. Isolates of *A. dauci* were not evaluated by Pryor and Gilbertson (2002).

The source of origin of the *A. petroselini* isolate identified in this study is not known, particularly since this isolate was not pathogenic on carrot foliage. Isolate Apt007 may have colonized carrot seed during seed cleaning and handling, or from windblown inoculum dispersed from infested parsley plants growing nearby carrots being harvested for seed production of this PI in Iowa. The USDA NPGS NCRPIS maintains seed of PI collections of various Apiaceae, including carrot and parsley (http://www.ars.usda.gov/main/site_main.htm?modecode=36-25-12-00).

The foliar pathogenicity tests in this study revealed some intraspecific variation in virulence among isolates at 4 wpi on both carrot and parsley foliage. The carrot PI lines evaluated were collected from all over the world, so it is possible these *Alternaria* isolates originated from the sites where the plants or seed were collected, the isolates may have infested the PI seed during seed increases by the NCRPIS in Ames, IA, from local sources of inoculum or other places where the carrot PI seed had been increased for the USDA NPGS (e.g., commercial seed companies sometimes increase seed of PI lines). Therefore, it is possible that variation in virulence among the pathogenic *Alternaria* spp. isolated from seed of these PI lines may reflect variation among the 66 carrot PI lines evaluated in the *X. hortorum* pv. *carotae* resistance screening (see Chapter 2), but this cannot be determined from the very limited number of isolates tested. Nonetheless, if infested seed of these carrot PI lines are grown in commercial carrot production areas, this could introduce genetic variability to *A. carotiincultae*, *A. dauci*, and *A. radicina* populations in areas where the seed is planted, which could make management of these pathogens more difficult for carrot growers if the introduced isolates are more virulent than existing, endemic populations of the species, as recorded for some pathogens in other crop species. For example, tomato late blight re-emerged as a significant cause of losses in the U.S.

in 1994 because of the widespread and national distribution of tomato plants infested with *P. infestans* to gardeners across the U.S. from major retailers (Fry and Goodwin, 1997).

Seedborne fungal pathogens have been reported on seed and vegetative propagative material of other agricultural crops maintained by the USDA NPGS, e.g., garlic cloves, and pea and spinach seed (Dugan et al., 2007; Kaiser and Hannan, 1986; Villarroel-Zaballos et al., 2012). Villarroel-Zaballos et al. (2012) demonstrated that *V. dahliae* on seed of spinach PIs caused symptoms on non-inoculated plants in a greenhouse screening for resistance to *V. dahliae*. Similarly, in the *X. hortorum* pv. *carotae* resistance screening, pathogenic *Alternaria* spp. on seed of carrot PIs caused damping-off on carrot seedlings, and *A. radicina* isolates subsequently caused rotting of carrot roots during or after vernalization (see Chapter 2).

For curators of the USDA NPGS, management of fungal pathogens such as these *Alternaria* spp. on plants of carrot PIs grown for seed production (e.g., with fungicide applications, planting pathogen-free seed, removal or incorporation of crop debris into soil, reducing leaf wetness, and rotating growing areas to nonumbelliferous crops), sterilizing harvest and seed cleaning equipment between seed lots, and monitoring seed and plants of carrot PIs for the presence of pathogenic *Alternaria* spp. when new accessions are acquired from various areas of the world, will be important to minimize the risk of distributing pathogen-infested carrot seed (Pryor, 2002; Pryor and Strandberg, 2002). Recipients of carrot PI seed could treat the seed chemically (e.g., with fungicides such as azoxystrobin, fludioxonil, iprodione, and/or thiram) or with hot-water or disinfectants to reduce spread and seed transmission of pathogenic *Alternaria* spp. on the PI seed (Abawi and Ludwig, 2003; Farrar et al., 2004; Lockhart and Delbridge, 1974). This should facilitate effective utilization of the carrot PI collection while reducing the risks associated with distributing seedborne *Alternaria* spp. that are pathogenic on carrot.

3.5. Literature Cited

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Table 3.1. Detection of *Alternaria dauci* and *A. radicina* on carrot seed of each of 66 Plant Introduction (PI) lines[a]

		% Seed infested[d]	
Line[b]	**Origin[c]**	***A. dauci***	***A. radicina***
PI 163238	India	0	4
PI 164136	India	0	36
PI 169482	Turkey	0	0
PI 169488	Turkey	100	28
PI 174206	Turkey	24	4
PI 174828	India	0	4
PI 175132	India	4	0
PI 175715	Turkey	0	16
PI 175716	Turkey	0	0
PI 175718	Turkey	0	8
PI 175719	Turkey	0	0
PI 176557	Turkey	0	0
PI 176558	Turkey	52	4
PI 176559	Turkey	0	0
PI 176969	Turkey	0	0
PI 177381	Turkey	32	4
PI 180834	Turkey	20	40
PI 181052	Pakistan	4	0
PI 181766	Lebanon	0	0
PI 181767	Lebanon	8	0
PI 182204	Turkey	0	12
PI 182207	Turkey	48	4
PI 183401	India	0	4
PI 187237	Belgium	20	4
PI 207480	Afghanistan	0	0
PI 220014	Afghanistan	12	0
PI 222723	Iran	48	4
PI 223360	Iran	12	4
PI 223777	Afghanistan	16	8
PI 226636	Iran	8	0
PI 234621	South Africa	0	4
PI 249535	Spain	0	12
PI 251228	Afghanistan	16	80
PI 261614	Spain	8	8
PI 263601	France	32	0
PI 264236	France	8	0
PI 264237	France	0	0
PI 264543	Japan	0	0
PI 271471	India	0	20
PI 274909	Turkey	8	4

Table 3.1. *continued…*

Line[b]	Origin[c]	% Seed infested[d]	
		A. dauci	***A. radicina***
PI 277710	Netherlands	8	28
PI 280706	Chile	0	4
PI 284773	Sweden	4	0
PI 288458	India	0	0
PI 288461	India	4	28
PI 294084	Japan	0	0
PI 326010	Tajikstan	4	0
PI 326011	Lithuania	0	4
PI 344072	Turkey	0	0
PI 344447	Iran	60	0
PI 357984	Former Serbia and Montenegro	96	0
PI 390885	Israel	0	8
PI 390889	Israel	4	8
PI 390893	Israel	68	4
PI 390900	Israel	24	4
PI 390901	Israel	12	0
PI 418967	China	12	48
PI 430524	Azerbaijan	8	12
PI 390887	Israel	36	8
PI 478863	Germany	0	4
PI 478873	Italy	0	0
PI 478883	France	4	0
PI 432900	China	0	28
PI 432905	China	0	0
PI 432906	China	0	0
PI 436674	China	0	4

[a] Seed assays were carried out using the malt agar protocols for detection of *Alternaria dauci* and *A. radicina* on *Daucus carota* seed as published by the International Seed Testing Association (2013b and 2013d).

[b] Seed of carrot PI lines were obtained from the United States Department of Agriculture (USDA) National Plant Germplasm System.

[c] PI lines were collected in various countries and are maintained at the USDA North Central Regional Plant Introduction Station in Ames, IA.

[d] Percentage of seeds infested with *A. dauci* or *A. radicina*, out of 25 seeds assayed/PI line.

Table 3.2. Fungal isolates obtained from seed of carrot (*Daucus carota*) Plant Introduction (PI) lines, with the species determined by sequencing the Alt a1 amplicon produced by PCR assay

Isolate	Species[a]	Carrot line[b]	Genbank accession number[c]
Acn010	*Alternaria carotiincultae*	PI 418967	KJ733005
Adc011	*A. dauci*	PI 169488	KJ732976
Adc013	*A. dauci*	PI 175132	KJ732977
Adc018	*A. dauci*	PI 220014	KJ732982
Adc020	*A. dauci*	PI 223360	KJ732983
Adc022	*A. dauci*	PI 261614	KJ732987
Adc031	*A. dauci*	PI 263601	KJ732989
Adc033	*A. dauci*	PI 263601	KJ732988
Adc035	*A. dauci*	PI 390901	KJ733003
Adc036	*A. dauci*	PI 177381	KJ732978
Adc037	*A. dauci*	PI 177381	KJ732979
Adc038	*A. dauci*	PI 390887	KJ732997
Adc039	*A. dauci*	PI 390893	KJ732998
Adc040	*A. dauci*	PI 390893	KJ732999
Adc041	*A. dauci*	PI 390893	KJ733000
Adc042	*A. dauci*	PI 390900	KJ733001
Adc043	*A. dauci*	PI 418967	KJ733007
Apt007	*A. petroselini*	PI 280706	KJ732992
Ard018	*A. radicina*	PI 164136	KJ732975
Ard024	*A. radicina*	PI 249535	KJ732985
Ard027	*A. radicina*	PI 182204	KJ732980
Ard029	*A. radicina*	PI 182204	KJ732981
Ard032	*A. radicina*	PI 223360	KJ732984
Ard035	*A. radicina*	PI 261614	KJ732986
Ard038	*A. radicina*	PI 271471	KJ732990
Ard039	*A. radicina*	PI 271471	KJ732991
Ard041	*A. radicina*	PI 288461	KJ732993
Ard043	*A. radicina*	PI 288461	KJ732994
Ard044	*A. radicina*	PI 326011	KJ732995
Ard045	*A. radicina*	PI 390887	KJ732996
Ard047	*A. radicina*	PI 390900	KJ733002
Ard050	*A. radicina*	PI 418967	KJ733004
Ard051	*A. radicina*	PI 418967	KJ733006
Ard054	*A. radicina*	PI 432900	KJ733008
Ard056	*A. radicina*	PI 432900	KJ733009
Ard059	*A. radicina*	PI 436674	KJ733010

[a] Fungal isolates obtained from carrot seed of PI lines were identified using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool for the Alt a1 sequences produced by PCR assay (Hong et al., 2005; Park et al., 2008).

[b] Isolates were obtained from seed of carrot PI lines from the United States Department of Agriculture National Plant Germplasm System using the malt agar seed assay protocols for detection of *Alternaria dauci* and *A. radicina* on *D. carota* seed (International Seed Testing Association, 2013b and 2013d).

[c] GenBank Accession number for the consensus Alt a1 DNA sequence produced by PCR assay for each *Alternaria* isolate.

Table 3.3. Pathogenicity test on carrot root discs of isolates of *Alternaria radicina* and related species obtained from seed of carrot Plant Introduction (PI) lines from the United States Department of Agriculture National Plant Germplasm System[a]

Isolate[b]	Species[c]	Rating[d]
Acn010	*A. carotiincultae*	6.0 ± 1.7
Adc038	*A. dauci*	1.0 ± 0.0
Apt007	*A. petroselini*	3.0 ± 0.0
Ard018	*A. radicina*	7.0 ± 1.0
Ard021	*A. radicina*	5.0 ± 2.0
Ard024	*A. radicina*	7.0 ± 2.0
Ard025	*A. radicina*	7.0 ± 1.0
Ard027	*A. radicina*	8.0 ± 1.0
Ard029	*A. radicina*	6.0 ± 1.7
Ard032	*A. radicina*	5.0 ± 1.0
Ard035	*A. radicina*	5.0 ± 1.0
Ard038	*A. radicina*	4.0 ± 1.0
Ard039	*A. radicina*	7.0 ± 1.0
Ard041	*A. radicina*	7.0 ± 1.0
Ard042	*A. radicina*	4.0 ± 1.0
Ard043	*A. radicina*	6.0 ± 1.7
Ard044	*A. radicina*	4.0 ± 1.0
Ard045	*A. radicina*	4.0 ± 1.0
Ard046	*A. radicina*	4.0 ± 1.0
Ard047	*A. radicina*	7.0 ± 2.0
Ard049	*A. radicina*	3.0 ± 0.0
Ard050	*A. radicina*	5.0 ± 1.0
Ard051	*A. radicina*	5.0 ± 1.0
Ard053	*A. radicina*	7.0 ± 1.0
Ard054	*A. radicina*	7.0 ± 1.0
Ard056	*A. radicina*	7.0 ± 2.0
Ard058	*A. radicina*	6.0 ± 1.7
Ard059	*A. radicina*	6.0 ± 0.0
BMP 0047	*A. radicina*	4.0 ± 1.0
BMP 0062	*A. radicina*	5.0 ± 1.0
BMP 0132	*A. carotiincultae*	4.0 ± 1.0
Water	Control	0.0 ± 0.0

[a] The experiment was set up with 3 replicate carrot discs inoculated/isolate in a completely randomized design, as described in the main text. The Kruskal-Wallis test (Spurrier, 2003) of the effect of fungal isolate on ranked carrot disc rot ratings was not significant ($P = 0.0846$). The non-inoculated control data were omitted from the statistical analysis.

[b] Each fungal isolate was obtained from seed of a carrot PI line during a malt agar seed assay as described in the main text. BMP 0047, 0062, and 0132 are *A. radicina*, *A. radicina,* and *A. carotiincultae* isolates, respectively, from Dr. Barry Pryor's program at the University of Arizona.

[c] Isolates were identified using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool for the Alt a1 sequences produced by PCR assay (Hong et al., 2005; Park et al., 2008).

[d] Mean ± standard error carrot disc rot based on a 0-to-9 scale, where: 0 = carrot disc with no discoloration; 1 = disc with slight discoloration; 2 = disc with tissue softening and necrosis; 3 = disc rotted slightly on the surface and edges, and on which sporulation typical of *A. radicina* was observed microscopically; 6 = disc rotted moderately with part of the disc succumbing to soft rot, and conidia typical of *A. radicina* observed on the disc; and 9 = disc rotted entirely with conidia typical of *A. radicina* observed microscopically.

Table 3.4. Pathogenicity test on foliage of carrot and parsley plants of *Alternaria* spp. isolated from seed of carrot Plant Introduction (PI) lines from the United States Department of Agriculture National Plant Germplasm System[a]

Isolate[b]	Species	Carrot disease severity 4 wpi (%)[c]	Parsley disease severity 4 wpi (%)[d]
Acn010	*A. carotiincultae*	1.75 abc	0.00 c
Adc013	*A. dauci*	1.00 bc	-
Adc041	*A. dauci*	4.50 a	0.25 bc
Apt007	*A. petroselini*	0.00 c	5.00 a
Ard038	*A. radicina*	1.88 ab	-
Ard039	*A. radicina*	3.00 ab	-
Ard049	*A. radicina*	4.25 ab	0.25 bc
Ard059	*A. radicina*	1.75 abc	-
BMP 0047	*A. radicina*	5.25 a	-
BMP 0132	*A. carotiincultae*	2.00 ab	-
BMP 0134	*A. carotiincultae*	4.25 ab	-
BMP 0142	*A. petroselini*	-	0.75 b
BMP 0144	*A. petroselini*	-	3.00 a
BMP 3127	*A. dauci*	1.00 bc	-
Water	Control	0.25	0.25

[a] The carrot foliar pathogenicity test was set up with five plants (cultivar Big Sur, Nunhems USA, Inc.) inoculated in each of four replicate pots in a greenhouse with three isolates of *A. carotiincultae*, three isolates of *A. dauci*, one isolate of *A. petroselini*, and five isolates of *A. radicina* in suspensions of 2.0 x 10^3 conidia/ml (except Adc013 which was inoculated at 1.1 x 10^3 conidia/ml) in a randomized complete block design, as described in the main text. The parsley foliar pathogenicity test was set up with five plants (cultivar Titan, Bejo Seeds, Inc.) inoculated in each of two replicate pots in a greenhouse with one isolate of *A. carotiincultae*, one isolate of *A. dauci*, one isolate of *A. radicina*, and three isolates of *A. petroselini* in suspensions of 2.0 x 10^3 conidia/ml in a randomized complete block design, as described in the main text.

[b] Each fungal isolates was obtained from seed of a carrot PI line in a malt agar seed assay as described in the main text. BMP 0047, BMP 0132, BMP 0134, BMP 0142, BMP 0144, and BMP 3127 (*A. radicina*, *A. carotiincultae*, *A. carotiincultae*, *A. petroselini*, *A. petroselini*, and *A. dauci*, respectively) are from Dr. Barry Pryor's program at the University of Arizona. The non-inoculated control data were omitted from statistical analysis.

[c] Mean percentage carrot foliage with lesions 4 weeks post inoculation (wpi) for five plants in each of four replicate pots. Means were log-transformed and compared using Fisher's protected least significant difference (LSD). Means with at least one letter in common were not significantly different ($P \leq 0.05$).

[d] Mean percentage parsley foliage with lesions 4 wpi for five plants in each of two replicate pots. Means were ranked and ranks compared using Fisher's protected least significant difference (LSD). Means with at least one letter in common were not significantly different ($P \leq 0.05$).

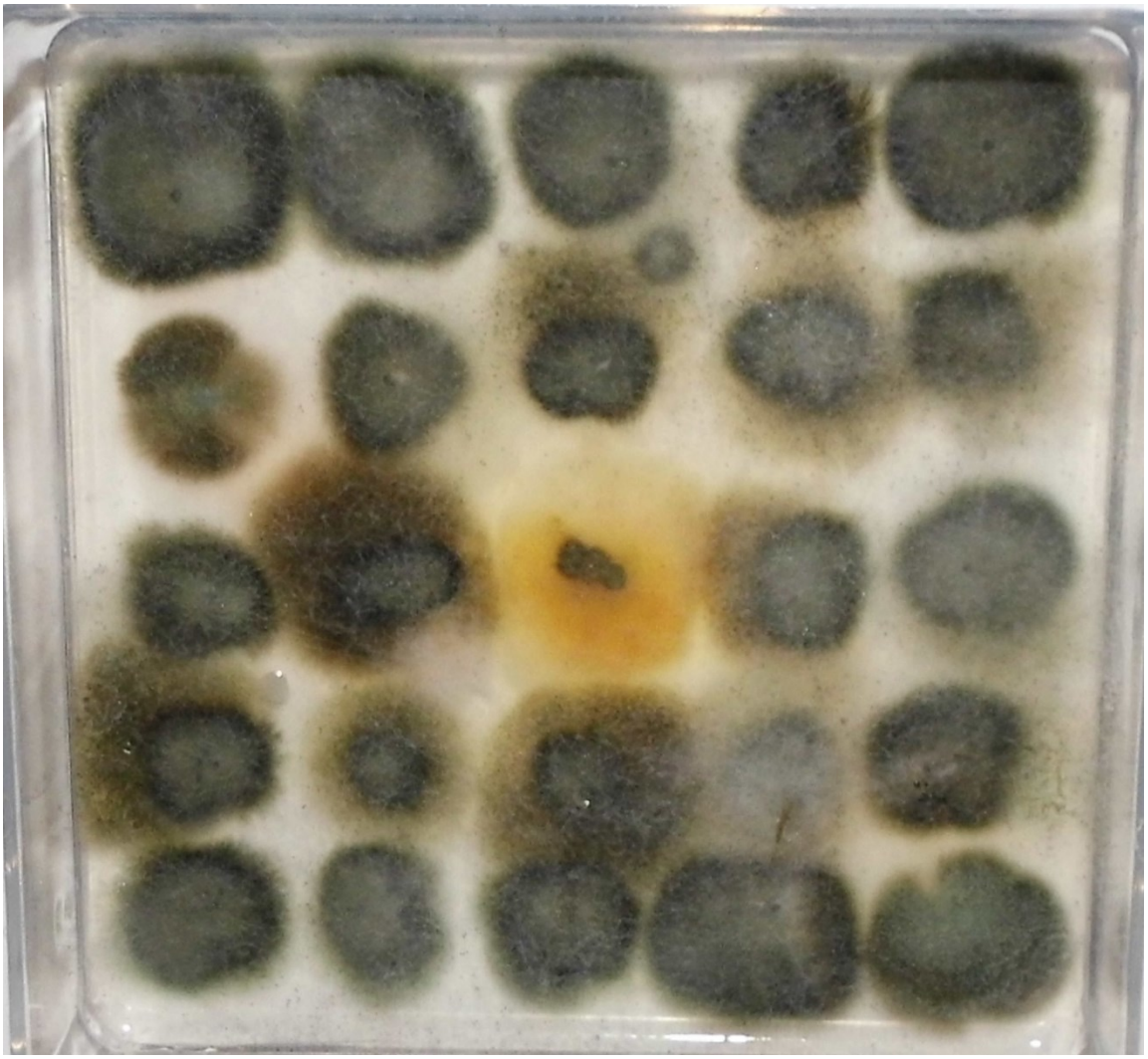

Fig. 3.1. Fungal growth observed 10 days after placing carrot seed on malt agar in accordance with the International Seed Testing Association's protocol for testing carrot seed for the presence of *Alternaria dauci* and *A. radicina* (ISTA 2013b and 2013d). Twenty-five seeds from each of 66 carrot Plant Introduction (PI) lines from the United States Department of Agriculture National Plant Germplasm System were placed on malt agar in an 11.0 cm x 11.0 cm x 2.9 cm box (one PI assayed/box). Seed in the boxes were examined at x40 to x100 magnification, and single-spore isolates were obtained by removing a conidium typical of *A. dauci* or *A. radicina* from carrot seeds, and plating each conidium onto chloramphenicol amended potato dextrose agar for subsequent species verification. Microscopic examination of the dark mycelium growing from each seed was associated with conidia typical of *A. alternata*. The seed surrounded by yellow-pigmented agar (third row and third seed from the left) was not associated with an *Alternaria* spp.

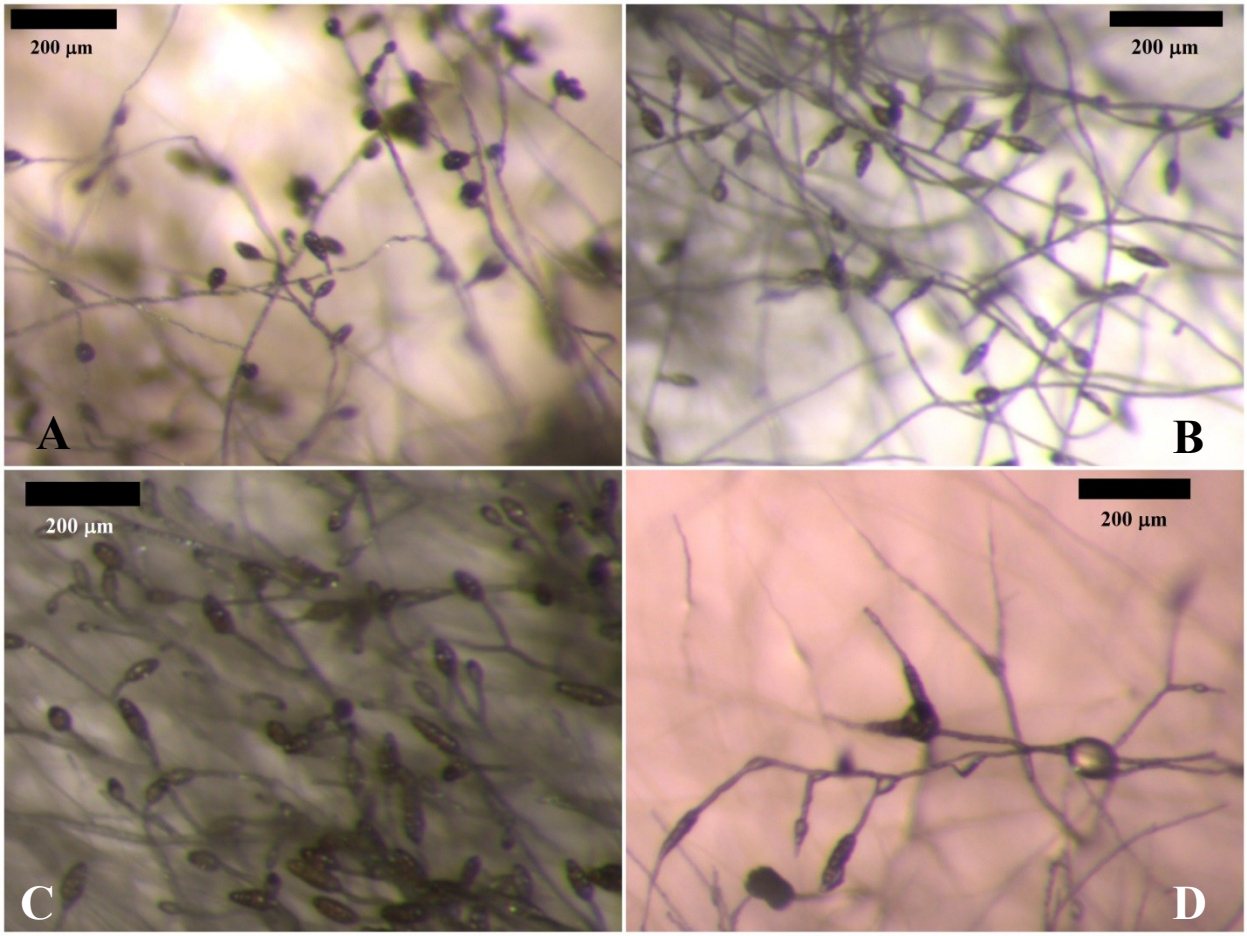

Fig. 3.2. Mycelia and conidia of isolates of *Alternaria* spp. obtained from seed of carrot Plant Introduction (PI) lines and grown on chloramphenicol amended potato dextrose agar for ≥10 days, magnified x40 to x100. Conidia of A) *A. carotiincultae* isolate Acn010, primarily solitary on conidiophores on aerial mycelium; B) *A. radicina* isolate Ard049 with conidia solitary or in groups of two to three/conidiophore on aerial mycelia; C) *A. petroselini* isolate Apt007 conidia; and D) *A. dauci* isolate Adc041 with solitary conidia.

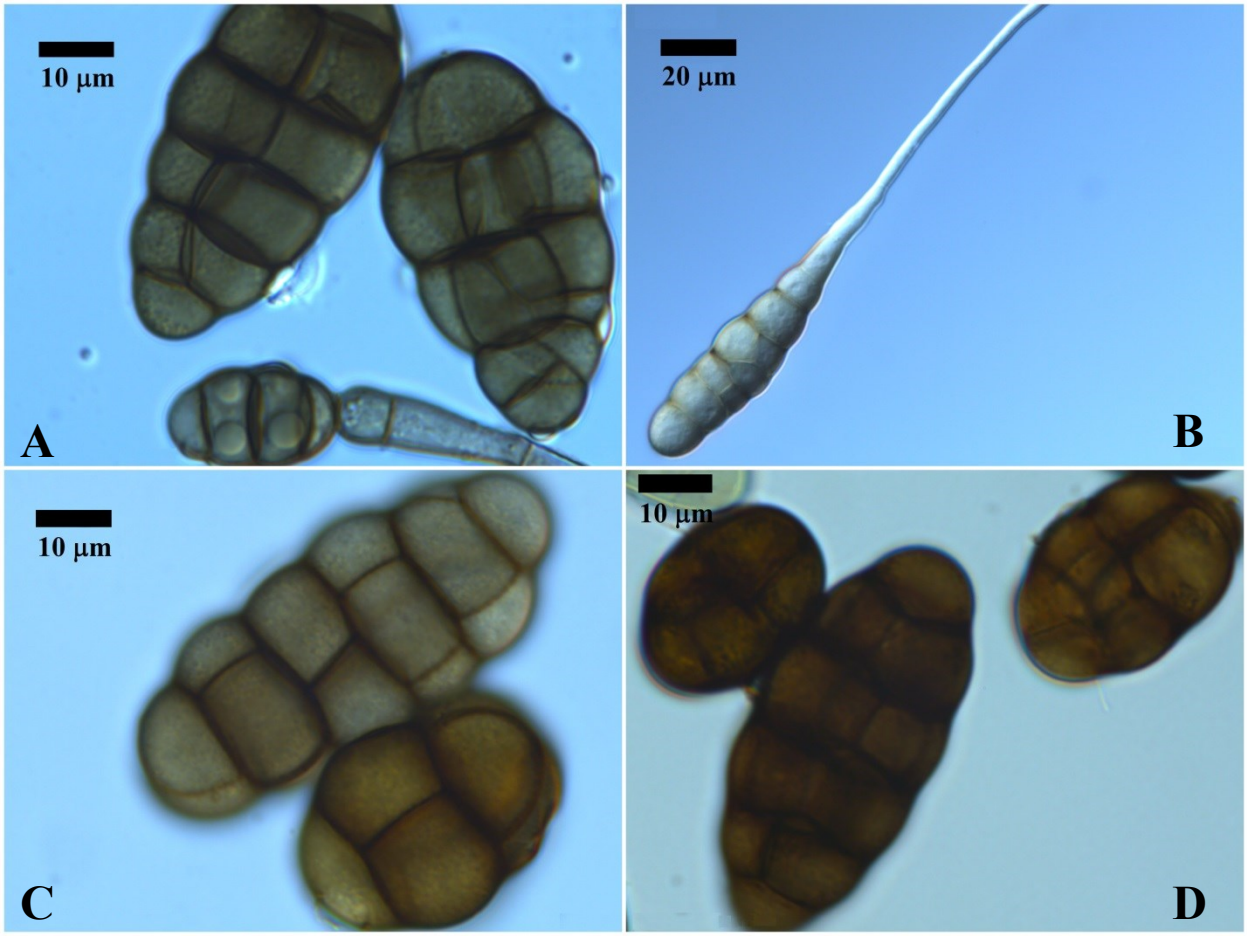

Fig. 3.3. Conidia of isolates of *Alternaria* spp. obtained from seed of carrot Plant Introduction lines and grown on chloramphenicol amended potato dextrose agar for ≥10 days. Conidia of A) *A. carotiincultae* isolate Acn010, B) *A. dauci* isolate Adc041, C) *A. petroselini* isolate Apt007, and D) *A. radicina* isolate Ard049.

Fig. 3.4. Symptoms on carrot leaves and petioles 4 weeks following inoculation of carrot foliage of the cultivar Big Sur (Nunhems USA, Inc.), with five plants inoculated in each of four replicate pots/isolate using a suspension of 2 x 10^3 conidia/ml of: A) *Alternaria carotiincultae* isolate Acn010, B) *A. dauci* isolate Adc041, C) *A. radicina* Ard049, and D) *A. dauci* isolate BMP 3127 in a greenhouse trial, as described in the main text.

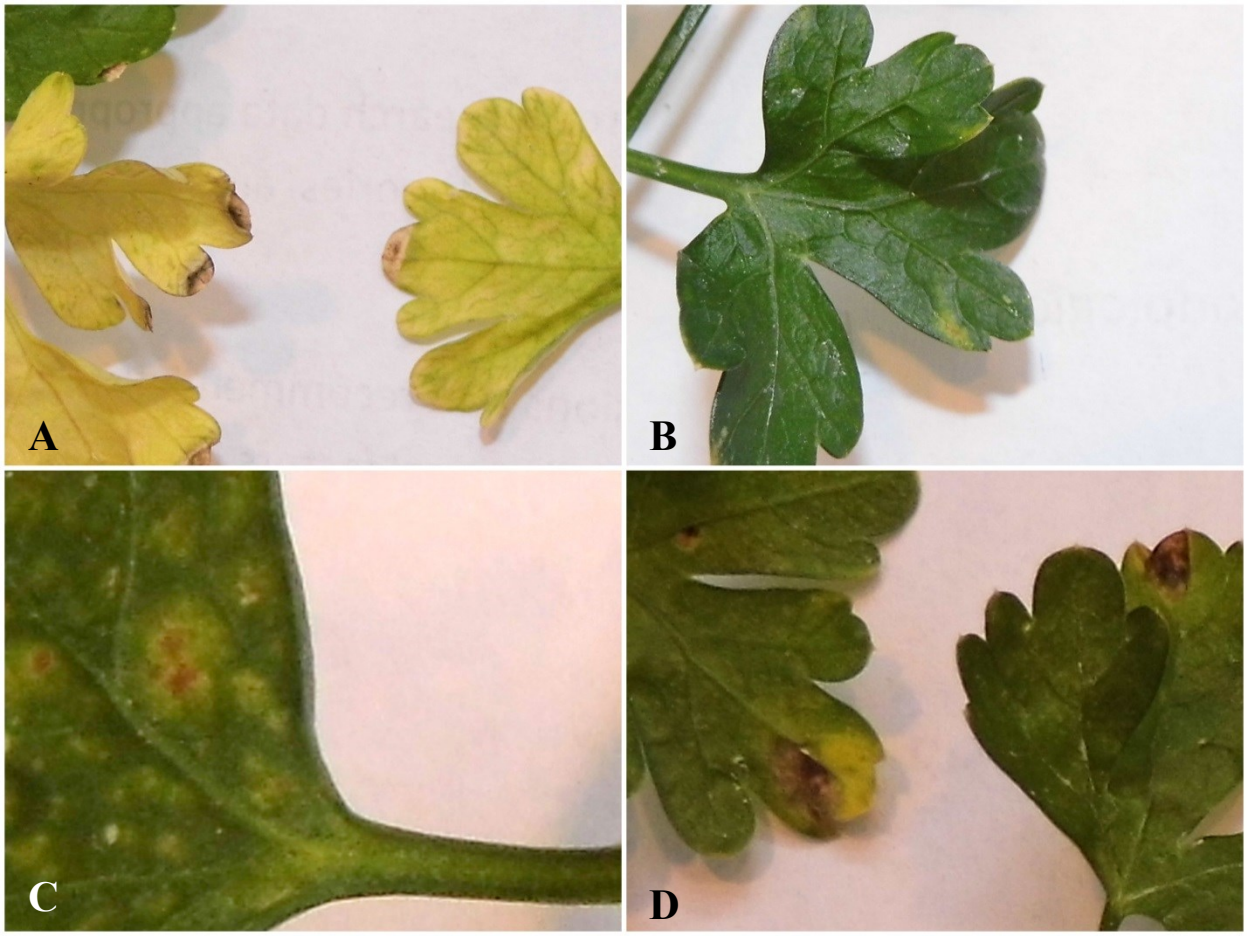

Fig. 3.5. Leaf blight and chlorosis on parsley foliage 4 weeks following inoculation of parsley foliage of the cultivar Titan (Bejo Seeds, Inc.), with five plants inoculated in each of two replicate pots/isolate in a greenhouse trial, using a suspension of 2 x 10^3 conidia/ml of: A) *Alternaria petroselini* isolate BMP 0142, B) *A. dauci* isolate Adc041, C) *A. radicina* isolate Ard049, and D) *A. petroselini* isolate Apt007, as described in the main text.

Chapter 4

Conclusions

Toward the start of this research, following lengthy discussions with my advisors and my venture into the limited literature on the topic, I learned that management of *Xanthomonas hortorum* pv. *carotae* on carrot can be problematic for growers, particularly carrot seed growers. *X. hortorum* pv. *carotae* is a stealthy pathogen that can be present in significant populations on symptomless carrot foliage. Thus, the inspiration for this research stemmed from the need for control measures that do not rely on monitoring and chemical control of the disease, e.g., disease resistance. The sparse literature on carrot genetic resistance to *X. hortorum* pv. *carotae* led me to believe that, perhaps, resistance to bacterial blight may not be an easily achievable goal in carrot. Past research indicated there are genetic differences in susceptibility to bacterial blight among carrot cultivars, yet no carrot germplasm has suppressed bacterial blight development to the extent of being effective as a management tool.

Later, I learned from Nunhems USA, Inc. plant pathologist, Dr. Peter Rogers, that the lack of available resistance to *X. hortorum* pv. *carotae* had less to do with overall carrot germplasm genetic potential and more to do with the complexities of the carrot seed industry. Generally, *X. hortorum* pv. *carotae* is not typically a significant problem for carrot root growers. This pathogen is seedborne, so carrot growers want to purchase carrot seed that is pathogen-free. For this reason, the responsibility to deliver clean seed falls on seed companies. Most *X. hortorum* pv. *carotae*-infested seed lots can largely be remedied of bacterial infestation using hot water treatment, so the demand for resistant cultivars is limited among carrot root growers. However, hot water treatment is time and resource intensive, and can reduce the shelf life of

seed. In addition, *X. hortorum* pv. *carotae* has been a persistent and growing problem in carrot seed production in the inland Pacific Northwest, an area that produces ≥ 80% of the U.S. carrot seed crop. The goal of this project was to quantify the pathogen and assess disease severity on carrot foliage of a subset of Plant Introduction (PI) lines from the United States Department of Agriculture (USDA) National Plant Germplasm System (NPGS), as well as cultivars from several seed companies, and two inbred male sterile lines from the USDA Agricultural Research Service (ARS), to identify PIs with resistance to *X. hortorum* pv. *carotae.* The ultimate objective is to incorporate any resistance identified in the PIs into commercially acceptable, public carrot breeding lines.

The resistance screening results from both 2012 and 2013 (Chapter 2) show significant differences in bacterial blight ratings and *X. hortorum* pv. *carotae* populations that developed on foliage 6 weeks post-inoculation among the various carrot genotypes. Some carrot PI lines (particularly PI 418967) appeared to limit bacterial blight symptoms and bacterial population development on foliage. The results suggest that there may be effective, quantitative resistance to *X. hortorum* pv. *carotae* in *Daucus carota* germplasm. The more resistant PIs identified could be used to develop bacterial blight-resistant parent lines that could add another management option for carrot seed companies and growers. Additionally, highly significant correlations were detected between severity of bacterial blight symptoms and *X. hortorum* pv. *carotae* populations recovered from the foliage in both 2012 and 2013. This information may be helpful for carrot breeders wishing to expedite carrot selections for resistance to the bacterial blight pathogen, since recovering *X. hortorum* pv. *carotae* populations from carrot foliage is time-consuming and expensive, especially when screening large quantities of plants. However, for comparing small differences in resistance to *X. hortorum* pv. *carotae* among a smaller set of carrot lines,

quantifying pathogen populations on foliage may be a more effective option to identify the most resistant lines.

While conducting this research to identify resistance to bacterial blight among carrot PIs, damping-off symptoms appeared on seedlings during the 2012 *X. hortorum* pv. *carotae* resistance screening. Putative pathogenic *Alternaria* spp. were isolated from damped-off carrot seedlings (see Chapter 3). Carrot seed from the USDA NPGS were demonstrated to be the source of these fungi. Following isolation of putative *Alternaria* spp. from carrot PI seed, identification of the species of these isolates using the Alt a1 PCR assay as well as pathogenicity tests, inter- and intraspecific variation in pathogenicity of the *Alternaria* isolates from PI seed were detected. *A. carotiincultae*, *A. dauci*, and *A. radicina* were isolated from the seed, in addition to one isolate of *A. petroselini*, a pathogen on parsley, but not carrot, obtained from seed of PI 280706. A subset of the isolates tested for pathogenicity on carrot and parsley foliage differed in ability to cause disease or severity of foliar symptoms, and a limited set of *A. radicina* and *A. carotiincultae* isolates all were able to cause black rot on carrot root discs. For curators of the USDA NPGS and those that receive seed of this germplasm collection, knowledge of the potential for carrot PI seed to be infested with these carrot pathogens, and appropriate management practices such as the use of seed treatments will help prevent the spread of these pathogens of carrot PI seed, and enable recipients of that seed to exploit the germplasm effectively as a genetic resource.