Assessing Susceptibility of Carrot Roots to Bacterial Soft Rot

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Abstract. Four methods for screening carrot (*Daucus carota* L.) germplasm for resistance to bacterial soft rot were compared. There were differences in resistance among strains, with most severe damage caused by *Erwinia carotovora* pv. *carotovora* SR 394 (L.R. Jones) Holland and *Erwinia carotovora* pv. *atroseptica* SR 159 (van Hall) Jenkinson. Inoculation of cross-sectional root slices with bacteria applied in suspension-soaked paper disks produced the most consistent response. The severity of disease damage was proportional to bacterial suspension concentration. With the development of a standard screening method, it may be possible for breeders to breed carrots with reduced susceptibility to soft rot.

Erwinia carotovora is the causal agent of soft rot of carrot. This disease causes severe root losses of carrot, mainly during post-harvest storage, but also in the field late in the growing season. The soft-rot bacterium is especially destructive on carrot at high temperature and high humidity (Segall and Dow, 1973). The soft-rot *Erwinia* spp. occur worldwide and are usually present in soil and water. They may also be disseminated by insects (Phillips and Kelman, 1982). Because most soft-rot *Erwinia* spp. attack many crops and are not host-specific, it is not uncommon to isolate more than one species or

pathovar from a diseased plant (Perombelon and Kelman, 1980).

The highest losses caused by soft-rot *Erwinia* spp. occur on vegetatively reproduced plants, such as potato, carnation, and chrysanthemum (Otazu and Secor, 1981). Extensive research has focused on the soft rot/blackleg complex in potato (*Solanum tuberosum* L.). Little attention has been directed to bacterial soft rot in carrot. Our study eval-

uated different methods of inoculating carrots to establish a simple, reliable test for evaluating breeding materials for possible resistance to *Erwinia*.

Bacterial strains used were *Erwinia carotovora* pv. *carotovora* (Ecc SR 319 and SR 392) and *Erwinia carotovora* pv. *atroseptica* (Eta SR 159), provided by Arthur Kelman, Univ. of Wisconsin, Madison, and a new strain Ecc SR 394, isolated from rotted carrot roots grown on organic soil in Wisconsin. The latter strain was identified as *E. carotovora* pv. *carotovora* based on an α -methyl-glucoside-containing medium as outlined by Cuppels and Kelman (1974).

All bacterial strains were maintained as stock suspensions in distilled water at room temperature. Bacteria were transferred to casamino-peptone glucose (CPG) broth for inoculum preparation and incubated at 25C on a rotary shaker (100 rpm) for 24 h to obtain cells in the log phase of growth. Bacteria were collected by centrifugation at 10,000 $\times$ g for 10 min, then resuspended in sterile, deionized water. The concentration (colony-forming units per 1 ml of bacterial suspension, cfu/ml) was adjusted by optical density with a Bausch and Lomb spectrophotometer (Rochester, N.Y.) at 620 nm.

Genetic stocks tested in this experiment included inbred lines and open-pollinated

Table 1. Disease response of four *Erwinia carotovora* strains (Ecc SR) at three concentrations on carrot inbreds W234B and W261B.

Ecc SR	Carrot line	Inoculum concn (cfu/ml)		
		2.5 $\times$ 10 ⁸	5 $\times$ 10 ⁷	5 $\times$ 10 ⁵
		Disease score ²		
394	W 234B	3.6 ab	2.9 def	0.7 j
	W 261B	3.8 a	3.3 bc	1.6 h
159	W 234B	3.3 bc	2.6 ef	0.3 k
	W 261B	3.6 ab	3.2 cd	1.1 i
319	W 234B	2.8 ef	2.2 g	0.1 k
	W 261B	3.4 bc	3.3 bc	0.7 j
392	W 234B	2.0 g	1.3 hi	0.1 k
	W 261B	3.3 bc	3.1 cde	0.2 k

¹Mean of disease severity, where 0 = no decay, 5 = total decay.

²Mean separation among all values by Duncan's multiple range test, $P < 0.05$.

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Disease classes:

1. Disease classes for bacterial soft rot damage in carrot root slices as used to determine disease score.

cultivars. Except for storage and root part experiments described later, roots were grown on organic soil in commercial carrot fields in Wisconsin in the summer, harvested in August, and stored 1 to 3 weeks at 0 to 4°C until testing. Root samples to be tested for resistance to soft rot were washed with tap water, surface-sterilized with 0.05% sodium hypochlorite (NaOCl) for 40 min and 70% ethanol for 5 min, rinsed in sterile water, and allowed to air-dry.

Four methods of inoculation were compared: A) The crown portion (about one-third of the root) with petioles trimmed to 2 cm was used. Two holes, ≈ 1 mm in diameter $\times$ 2 cm deep, were made in the root cambium region through the cut surface of the crown portion. Then 10 μ l of *Erwinia* suspension was injected into the holes with a syringe, one strain per hole. B) Same as method A, except the middle third of the root was used. Inoculation was always on the distal cut root surface. C) Cross-sectional, 5-mm-thick slices of root were used. Disks of Whatman #2 filter paper (5 mm in diameter) were soaked in bacterial suspension for at least 30 min and then placed on the proximal cut surface of the carrot slice over the cam-

bium region. D) Root slices, 5 mm thick, were inoculated with 10 μ l of bacterial suspension placed on the freshly cut surface, without additional wounding.

After inoculation, carrot root samples were placed in plastic boxes (40 $\times$ 25 $\times$ 5 cm) lined with wet paper towel, sealed with plastic wrap, and placed in plastic bags. These inoculation chambers were incubated at 22°C for 48 to 96 h.

Soft-rot severity was determined on the basis of disease score, an estimate of the area decayed. A scale of 0 to 5 was used, where 0 = no decay and 5 = total decay (Fig. 1). To evaluate disease development for methods A and B, roots were cut through the point of inoculation after 96 h of incubation. Disease response for methods C and D was evaluated after 48 h.

Roots from one open-pollinated population (B951-1) and male-sterile and fertile (maintainer) counterparts of two inbred lines (B493 and B9304) were grown in Florida and California. Postharvest storage was at 4°C in paper bags within non-sealed plastic bags. Seven roots of each entry were evaluated 2, 6, and 12 weeks after harvest. All roots were intact through the first 2 weeks

of storage. Then, 14 roots were cut transversally at midsection and 14 left intact for each inbred line. These 28 roots of each stock were returned to storage. After 6 and 12 weeks of storage, seven each of the cut (both crown and top) and intact roots were screened. Root slices were inoculated (method C) with *E. carotovora* pv. *carotovora* SR 394 at a concentration of 5×10^7 cfu/ml inoculum.

Significant differences in disease development were found among strains of *Erwinia* on two carrot lines (Table 1). Ecc SR 394, freshly isolated from decaying carrots, produced the most severe tissue damage in this study, as it did on potatoes (Austin et al., 1988). Eta SR 159, isolated from potatoes, produced slightly less severe damage. Ecc strains SR 319 and SR 392 produced milder symptoms. Because of their greater pathogenicity, all subsequent experiments were done with Ecc SR 394 and Eta SR 159. All four *E. carotovora* strains at all concentrations incited a more severe response on carrot line W 261B than on W 234B. Differences in disease response among carrot lines were greater with the less pathogenic *Erwinia* strains and lower inoculum concentrations. There were no strain $\times$ line or strain $\times$

Table 2. Bacterial soft rot development in 15 carrot strains.^a

Strain	Disease rating for four methods of infection ^b			
	A	B	C	D
	<i>Expt. 1</i>			
W77C	0.5 ab ^c	0.5 b	2.0 b	2.5 b
W97B	1.0 a	2.3 a	3.0 a	2.8 b
W266C	0.8 a	1.6 a	3.2 a	3.3 a
WAYII	0.2 b	0.5 b	2.3 b	2.8 b
	<i>Expt. 2</i>			
F524		4.0 a	5.0 a	4.4 bc
B2302		2.5 b	4.2 b	4.3 cd
B5238		3.0 b	3.6 d	4.0 de
B5316		4.0 d	4.7 a	4.3 c
B9695		0.8 d	2.9 e	2.5 f
HCM		1.8 c	3.6 d	4.8 a
B493		2.8 b	3.8 cd	3.9 e
B9304		3.7 a	5.0 a	4.8 a
Imperial Long Scarlet		1.3 cd	4.0 bc	4.0 de
Waltham Hicolor		1.1 cd	3.9 bc	4.0 de
Scarlet Nantes		3.0 b	4.8 d	4.7 ab

^a*Erwinia* strain Ecc SR 159, 5×10^7 (cfu/ml) used in Expt. 1; strain Ecc SR 394, 2.5×10^8 cfu/ml in Expt. 2.

^bMean of disease severity, where 0 = no decay, 5 = total decay. Methods A, B, C, and D described in the text. No development in noninoculated controls on sterile water-inoculated controls with any method.

^cMean separation in columns by Duncan's multiple range test, $P < 0.05$. Experiments 1 and 2 were analyzed separately.

concentration interactions. Similar relationships among *Erwinia* spp., pathovars, and strains have been observed in potato (Lapwood et al., 1984).

Inoculum concentration effects were significant for a given carrot line and *Erwinia* strain in all cases, except for the two higher concentrations of the less pathogenic strains on the more susceptible carrot line. An inoculum concentration of 5×10^6 cfu/ml was also tested and found to induce disease severity comparable to the 5×10^7 cfu/ml concentration (data not presented).

Inoculation of carrot root slices with methods C and D resulted in a more severe disease response than when pathogen was injected into larger root sections using methods A and B (Table 2). Variation in response was also reduced with methods C and D. Inoculation with soaked paper disks (method C) produced a response similar to that from a surface drop of inoculum (method D). Reduced disease severity was observed with crown inoculation (method A) than with midroot inoculation (method B). Similar responses in soft-rot susceptibility have been reported in a comparison of apical and basal ends of potato tubers (Otazu and Secor, 1981). Methods B, C, and D gave similar rankings of disease response among four Univ. of Wisconsin inbreds, seven U.S. Dept. of Agriculture inbreds of diverse genetic background, and three open-pollinated varieties

(Table 2); however, HCM and B5238 were not consistent in their response. W77C and WAYII were most resistant by all methods of inoculation in Expt. 1, whereas B9695 was most resistant with all methods in Expt. 2.

Duration of postharvest storage had no effect on severity of tarot bacterial soft rot (data not presented). Results at 2, 6, and 12 weeks postharvest were similar, as has been observed in potato (Workman and Holm, 1984). Differences in response to *Erwinia* attack in potato depended on tuber storage condition before inoculation (Bartz and Kelman, 1984; Workman and Holm, 1984). In contrast, we noted that stored, intact and cut carrot roots yielded similar results. The root tip tended to be slightly more susceptible than the crown.

In choosing an *Erwinia* soft rot screening method suitable for a carrot breeding program, the paper disk method (method C) provides some advantages. When root slices are used to evaluate disease response of roots, the crown of the root (which is necessary for seed production) is not damaged, as would happen in method A, or in the method developed by Skadow (1978). Furthermore, several root slices can be obtained from a single root, enabling replicated screening from one plant. These use of a paper disk (method C) reduced drying of the bacterial suspension and maintained better conditions for infec-

tion and disease development. In wounding and droplet inoculation (methods D and E), which are similar to the method used by Lebeda (1985), evaporation was a problem, which may have reduced the disease development. The observed variation between carrot lines suggests the possibility for genetic improvement of resistance to *Erwinia* in carrot. However, correlation of laboratory response with damage realized either in storage or the field remains to be demonstrated. To ensure accurate comparisons, roots grown, harvested, and stored together need to be contrasted. Genetic differences in response to *Erwinia* infection between carrot strains may then be determined.

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