Incidence, Resistance, and Evaluation of Chemical Controls for Bacterial Soft Rot in Carrot Stecklings

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Introduction

Bacterial soft rot, caused by *Pectobacterium* spp. (formerly *Erwinia carotovora*), can be an important disease of certain carrot steckling lines used for hybrid carrot seed production. In surveys conducted in two hybrid Kuroda steckling-to-seed carrot seed fields in Jefferson County, OR during the summer of 2014, between 22 and 43% of female plants exhibited soft rot symptoms. A follow-up survey in 2015 observed between 19 and 27% of female plants with soft rot symptoms. It has also been observed that symptoms in the field are more severe on the female line compared the male line. The objectives of this study were to: 1) quantify the incidence of soft rot infection in stecklings used for hybrid seed production; 2) determine if the male line is more resistant to soft rot than the female line; and 3) test the efficacy of chemical treatments to reduce losses caused by soft rot.

Materials and Methods

Sampling for natural infection

Petioles from 100 each of male and female carrot stecklings were sampled from steckling cold storages in March and tested for the presence of soft rot bacteria. Non-surface sterilized sections of petioles 1 cm in length were sampled 1 cm away from the crown of each steckling. Depending on the number of petioles on each steckling, between one and eight pieces were placed in phosphate buffer. Petioles were incubated in phosphate buffer for 15 min at room temperature, vortexed, and a 10 µl aliquot was plated onto crystal violet pectate (CVP) agar.

Crowns and root tissue from a total of 100 each of male and female stecklings were sampled for the presence of soft rot bacteria. One half of the male and female stecklings were surface-sterilized in 10% bleach, rinsed with water, and air-dried. The other half of the stecklings were not surface-sterilized. A 1 cm slice of crown tissue was aseptically cut from each steckling and placed in a moist chamber. A second 1 cm slice of root, approximately 5 cm away from the top of the crown, was cut and also placed in a moist chamber. A slice of carrot was inoculated with 10 µl of a virulent *Pectobacterium* strain and placed in each chamber as a positive control. The carrot slices were incubated for 4 days at 82° F and the moist chambers were misted twice daily with sterile water.

Resistance assay

Slices (2 cm thick) were taken from the middle of 20 male and 20 female stecklings. The carrot slices were surface-sterilized in 10% bleach, air-dried, and weighed. A 10 µl drop of a virulent *Pectobacterium* strain was placed on each slice and placed in moist chambers in a completely

randomized design. Mock inoculations with phosphate buffer were included as negative controls (one male and one female per moist chamber). Carrot slices were incubated at 82° F and the moist chambers were misted twice daily with sterile water. After 6 days, the rotted tissue was washed from the slices. The slices were air dried and weighed to determine the amount of tissue lost due to soft rot. The experiment was repeated once.

Treatments for soft rot control in stecklings

A field trial was set up at the Central Oregon Agricultural Research Center to test the efficacy of treatments to reduce soft rot in stecklings. The trial consisted of 4 rows and two border rows. Plots 25 ft long were set up with 30 inch row spacing and five foot buffers in between plots. Borders 25 ft in length were planted at the end of each row. A total of 9 treatments (Table 1) and a non-treated control were arranged in a randomized complete block design. Drench treatments were applied and the stecklings were allowed to air-dry before planting. Stecklings were hand-planted approximately 6 inches apart in each row. In-furrow treatments were applied in a 1 ft band after planting using a CO_2 backpack sprayer. Stecklings were watered in using overhead irrigation and then drip-irrigated for the remainder of the season. Off-types were rogued throughout the season. Standard management practices for steckling-to-seed hybrid carrot seed crops were followed. Plots were evaluated every 3 to 4 weeks and the number of plants exhibiting soft rot symptoms was recorded. Plants with aboveground symptoms of soft rot (chlorosis, wilting, black discoloration on stems) were pulled from the ground and to verify that soft rot was present on belowground tissue (soft, wet, rotting stecklings).

Results and Discussion

Sampling for natural infection

Visual examination of the petioles suggested that most petioles on female stecklings were rotting, with only five petioles exhibiting a healthy appearance. Petioles of the majority of male stecklings appeared to be healthy. However, soft rot bacteria were recovered from 87% of petiole samples from male stecklings and 94% of petiole samples from female stecklings as indicated by pitting on CVP agar.

Approximately 8 and 87% of male and female crowns, respectively, exhibited natural infection with soft rot bacteria and approximately 5% of male and 78% female root slices also exhibited natural infection with soft rot bacteria (Table 2). Based on visual examination of stecklings, it appears that the infection begins at the crown, likely the petiole, and moves down the steckling over time (Fig. 1). This is consistent with samples brought to the plant pathology lab in the summer of 2014.

Resistance assay

Carrot root slices from female carrot lines exhibited significantly greater tissue loss compared to carrot root slices from male carrot lines in both trials (Fig. 2). Less than 3% of carrot slices from

male lines exhibited soft rot symptoms following artificial inoculations, while 95% of carrot slices from female carrot lines exhibited symptoms. Asymptomatic slices increased in mass, likely due to imbibing water during six days of incubation at >95% relative humidity.

Treatments for soft rot control in stecklings

Although some plants survived until the final assessments performed on July 29, the majority of plants (84 to 93%) exhibited soft rot symptoms on belowground tissues (Table 1). A significant difference was not observed among treatments ($P = 0.5$). Further research is needed to identify chemical control options for soft rot in steckling-planted carrot seed crops.

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Table 1. Percentage of healthy and diseased plants in experimental plots after treatments with bactericides and disinfectants

Treatment (application method)	Active ingredient (concentration)	Rate	Healthy plants[1]	Diseased plants[1]
Non-treated	**NA**	**NA**	4%	93%
ManKocide (in furrow spray)	**Mancozeb (15%), Cu(OH)2 (46.1%)**	**2.5 lb/acre**	6%	92%
Ocion PT81 (in furrow spray)	**CuSO4•5H2O (20.3%)**	**40 oz/acre**	15%	84%
Ocion FT33 (in furrow spray)	**Cu (4.16%), Zn (1.64%), S (4.97%)**	**40 oz/acre**	9%	89%
KleenGrow (in furrow spray)	**Didecyldimethyl ammonium chloride (7.5%)**	**25 oz/acre**	10%	86%
Oxidate (in furrow spray)	**Hydrogen dioxide, peroxyacetic acid**	**1 gal/acre**	8%	88%
Ocion PT81 (pre-plant drench)	**CuSO4•5H2O (20.3%)**	**0.4 oz/gal**	9%	90%
KleenGrow (pre-plant drench)	**Didecyl dimethyl ammonium chloride (7.5%)**	**1.5 oz/gal**	10%	89%
Oxidate (pre-plant drench)	**Hydrogen dioxide, peroxyacetic acid**	**1.28 oz/gal**	12%	84%
Bleach (pre-plant drench)	**NaOCl (8.25%)**	**10,000 ppm**	7%	91%

[1] Off-type stecklings were rogued and not included in the healthy or diseased plant counts.

Table 2. Incidence of natural soft rot infection in the crowns and roots of male and female carrot stecklings

		Non-surface-sterilized	Surface-sterilized	Mean infected
Male	**Crown**	13%	4%	**8%**
	Root	9%	2%	**5%**
Female	**Crown**	86%	88%	**87%**
	Root	80%	76%	**78%**

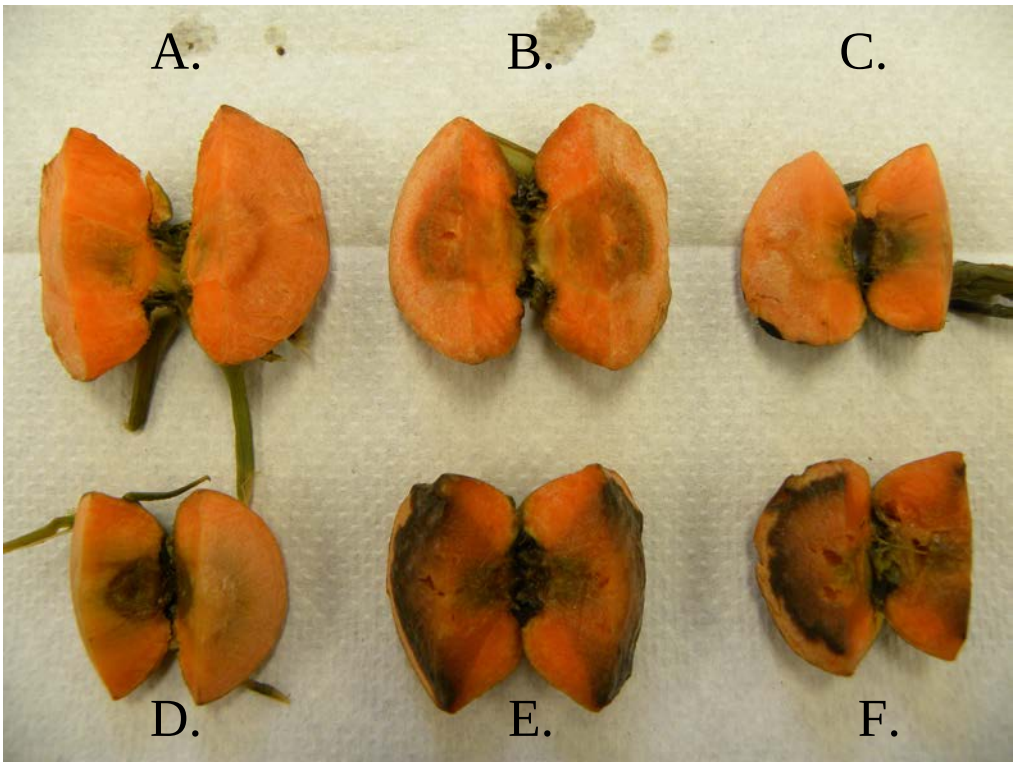

Figure 1. Soft rot progression from petioles into the crown. A) Healthy crown; B-F) Increasing severity of soft rot in the crown, likely originating from the petioles.

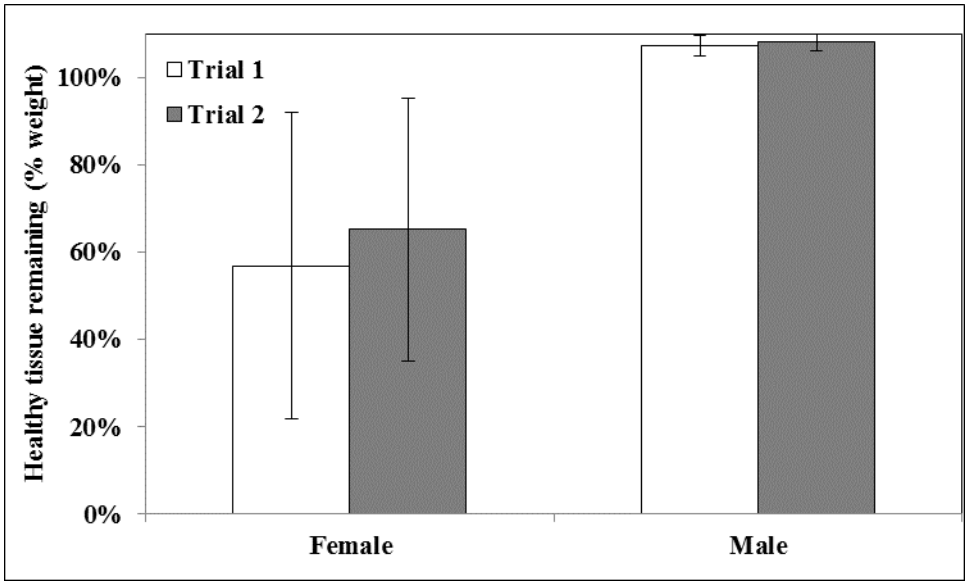

Figure 2. Percentage of healthy tissue remaining (by weight) after inoculating carrot root slices with soft rot *Pectobacterium*. Error bars indicate standard deviation values.