Influence of Soil Saturation and Temperature on *Erwinia chrysanthemi* Soft Rot of Carrot

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ABSTRACT

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In 1998, soft rot caused by *Erwinia chrysanthemi* resulted in an estimated loss of 1,800 tons of carrots in California. The disease appeared to be related to unusually high temperatures and excessive irrigation. To determine the optimum conditions for development of soft rot under controlled conditions, pots of carrots inoculated with *E. chrysanthemi* were saturated with water and incubated at 20, 25, 30, or 35°C. Plants were harvested and examined for disease 12, 24, 36, 48, 72, and 96 h after inoculation. Negligible disease occurred after 12 h. Disease severity and incidence increased with increasing temperature and duration of saturation from 24 to 96 h. In a second experiment, carrot disks were inoculated with three isolates each of *E. chrysanthemi* and *E. carotovora* subsp. *carotovora* and incubated at 15, 20, 25, 30, and 35°C. After 48 h, the disks were washed to remove rotted tissue and reweighed. Neither bacterium reduced carrot disk weight at 15°C. In general, moderate weight reduction occurred at 20 and 25°C. The greatest degree of soft rot was caused by *E. chrysanthemi* at 30 and 35°C. *E. carotovora* subsp. *carotovora* isolates were relatively less virulent than *E. chrysanthemi* at 30°C and none of the *E. carotovora* subsp. *carotovora* isolates reduced carrot disk weight at 35°C. This is the first report of *E. chrysanthemi* causing soft rot of carrot in California. Based on these results, growers should limit the length of time carrot roots are exposed to saturated soil, especially at high soil temperatures.

In August through October, 1998, a high incidence of soft rot occurred in carrot fields in Madera, Kern, and Imperial Counties, California. Numerous truckloads of harvested carrots were discarded and some fields were abandoned when the incidence of disease reached 25% or more. An estimated 1,800 tons of carrots were lost during this period. Soft rot lesions were pale orange, circular, 5 to 15 mm in diameter, and soft to the touch. There were multiple lesions per carrot root and some lesions coalesced to form large, irregular rotted areas. When infected carrots were washed at the packing sheds, soft rotted tissue was removed, creating craters in the carrots, resulting in an unmarketable product. It was hypothesized that high soil temperatures were associated with the high incidence of soft rot. Data from a CIMIS weather station maintained at the University of California Cotton Research Station, Shafter, CA, revealed that average daily maximum soil temperatures at a depth of 15 cm in August and September, 1998, were 28.9 and 26.6°C, respectively. These

averages are 2.2 and 2.4°C higher, respectively, than the averages for the previous 10 years in the San Joaquin Valley.

Little information is available on soft rot of carrot. *Erwinia carotovora* subsp. *carotovora* was identified as the causal agent of carrot soft rot in India (12) and Korea (2) after severe disease outbreaks. In Texas, both *E. chrysanthemi* and *E. carotovora* subsp. *carotovora* caused core rot of carrots in the field (16). In stored carrots, infection by *E. carotovora* increased with increasing storage temperatures up to 24°C, but declined rapidly at higher temperatures (9). High relative humidity during storage and wounding prior to storage also resulted in higher infection rates. In Florida, the addition of chlorine to wash water and chilling was found to significantly reduce carrot soft rot in storage (14). The goals of this study were to determine the cause of the epidemic in California in 1998 and examine the influence of temperature and soil saturation on its development.

MATERIALS AND METHODS

Isolation and identification of isolates.

Culled carrots with visible soft rot were obtained from packing sheds in Kern County. Cultures of bacteria were obtained by streaking a loopful of soft carrot tissue directly onto Miller-Schroth media (10). Orange-red colonies were transferred to yeast-dextrose-carbonate (YDC) agar after

the plates were incubated for 2 to 3 days in a 28°C incubator. Eleven isolates from five fields were identified by a profile for utilization of 95 carbon sources (Biolog GN Micro Plates, Hayward, CA), fatty acid methyl ester analysis (MIDI Microbial Identification System version 3.8, Newark DE), gram determination (3% KOH string test; 15), ability to soft rot potato disks, acid production from palatinose, methyl α -D-glucopyranoside, and trehalose, utilization of malonate, and phosphatase production (4). Three isolates were tested for erythromycin sensitivity. To test the ability of the isolates to cause soft rot, carrot disks were inoculated with seven isolates. Carrots were surface sterilized for 30 min in 0.525% sodium hypochlorite, rinsed with deionized water, and cut into disks with a sterilized knife. A loop of bacteria from a 24-h culture grown on YDC at room temperature was streaked onto the carrot disks. Sterile water was used as a control. Four disks were inoculated with each isolate. All disks were incubated in a plastic box lined with moist paper towels for 2 days at room temperature (19 to 21°C). Bacteria were then reisolated and reidentified as described above. In addition, six whole carrots were inoculated with each isolate. In these tests, carrots were surface sterilized, wounded by nicking the carrot with a sterilized scalpel, and inoculated with 10 μ l of a 10⁸ CFU/ml suspension. The concentration of the bacteria was determined by suspending a loopful of bacteria in sterile distilled water and adjusting the percentage transmittance at 500 nm to 20% on a spectrophotometer (Sequoia-Turner Model 340, Mountain View, CA). Sterile water was used as a control. Carrots were incubated in a plastic box lined with moist paper towels for 2 days at room temperature (19 to 21°C). Bacteria were then reisolated as described above.

Effect of temperature and soil saturation duration on soft rot caused by *E. chrysanthemi*. Carrot cv. Caro-Pak was planted in a potting mix (peat moss: vermiculite, 1:1) in 11-cm-diameter styrofoam cups (Dart Container Corp, Mason, MI) with four drain holes. Seedlings were thinned to 4 to 5 per cup at the three-true-leaf stage. Carrots were watered daily with deionized water and fertilized weekly with a dilute complete nutrient solution. When the carrot roots were 10 to 12 cm long and 1.5 to 2 cm wide at the shoulder, the cups

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were placed inside new cups, moved to 20, 25, 30, or 35°C water baths, and allowed to equilibrate for 12 h. Six cups were placed in each water bath for each replication. Following the addition of 20 ml of a 10^8 CFU/ml suspension of *E. chrysanthemi* to the top of each cup, the cups were saturated with deionized water. Water was added as necessary to the water baths to maintain the original water level and to cups to maintain saturation of the potting mix. Controls for each temperature were saturated, noninoculated cups and cups inoculated with *E. chrysanthemi* but not saturated. Root zone temperatures in the cups in the 20, 25, 30, and 35°C water baths were 20, 25, 29, and 33°C, respectively. One cup from each bath was removed 12, 24, 36, 48, 72, or 96 h after inoculation. At each sampling time, carrot roots were removed from the cups, rinsed, and rated for degree of infection using a pretransformed scale where 0 = 0%, 1 = 1 to 7%, 2 = 8 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, 5 = 76 to 93%, and 6 = 94 to 100% of the root surface with soft rot lesions. The average rating of all carrots in a cup was used in the data analysis. Each treatment consisted of three replications in time. The experiment was performed three times and the data combined. Data were analyzed with the analysis of variance (ANOVA) and REG procedures of SAS (SAS Institute, Cary, NC) and regression lines were compared with the REGR procedure of MSTAT-C (Michigan Statistics Group, East Lansing, MI).

Comparison of soft rot ability of *E. chrysanthemi* and *E. carotovora* subsp. *carotovora*. The ability of three isolates each of *E. chrysanthemi* and *E. carotovora* subsp. *carotovora* to cause soft rot of carrots at 15, 20, 25, 30, and 35°C was examined. Mature Caro-Pak carrots, har-

vested from a commercial field, were washed in running water, surface sterilized in 0.525% sodium hypochlorite for 30 min, rinsed with deionized water, and cut into disks with a sterilized knife. A small core, 4 mm in diameter and 2 to 3 mm deep, was removed from the center of each carrot disk with a flamed cork borer. Disks were then weighed and placed on damp paper towels in plastic boxes. A 50- μ l aliquot of a 10^8 CFU/ml suspension of each isolate from 24-h cultures on YDC was placed in the well in each carrot disk. Controls were inoculated with 50 μ l of sterile deionized water. Disks from each carrot were inoculated with each isolate and the control. The plastic boxes were placed in 15, 20, 25, 30, or 35°C incubators. After 48 h, the soft, rotted portion of each disk was washed out under running water. Disks were weighed again and the difference between initial and final weights calculated. This difference, the amount of carrot tissue consumed by the bacteria, was analyzed using the SAS ANOVA procedure. The experiment was performed three times and the data combined.

RESULTS

Isolation and identification of isolates. *E. chrysanthemi* was identified from all affected carrots based on the physiological tests: gram negative; soft rot of potato disks; utilization of malonate; positive for phosphatase production; sensitive to erythromycin; and negative for acid production from palatinose, glucopyranoside, and trehalose. The identity of *E. chrysanthemi* was confirmed by FAME analysis (similarity index 0.881 to 0.923) and the Biolog test (similarity index 0.623 to 0.808). Carrot disks and wounded whole carrots inoculated with *E. chrysanthemi* developed soft rot, whereas the controls did not.

Fig. 1. Influence of temperature and duration of soil saturation on soft rot of carrots. Carrots were grown in 11-cm-diameter styrofoam cups and were 10 to 12 cm long and 1.5 to 2 cm wide at the shoulder when inoculated. Severity of soft rot was estimated using a pretransformed scale where 0 = 0%, 1 = 1 to 7%, 2 = 8 to 25%, 3 = 26 to 50%, 4 = 51 to 75%, 5 = 76 to 93%, and 6 = 94 to 100% of the root surface with lesions. Each point represents the average of three replications in each of three trials, four to five carrots per replication. R^2 values for the 20, 25, 30, and 35°C treatments were 0.16, 0.30, 0.38, and 0.49, respectively.

Effect of temperature and soil saturation duration on soft rot caused by *E. chrysanthemi*. Temperature and duration of saturation significantly affected soft rot severity (Fig. 1). Little disease occurred during the first 24 h, regardless of temperature. After 24 h, the severity of rot increased with increasing temperature and duration of saturation. At 96 h, 75% of the surface area of carrots in the 35°C water bath was rotted, whereas only 7% was rotted in the 20°C water bath. There was a positive linear relationship between disease severity and duration of saturation for each temperature. Slopes of the regression lines at 35 and 30°C and slopes at 25 and 20°C were significantly different ($P = 0.02$ and 0.04, respectively). Slopes of the regression lines at 30 and 25°C were not significantly different ($P = 0.69$). Neither control treatment (saturated with water but not inoculated and inoculated but not saturated with water) developed soft rot.

Temperature and duration of saturation also significantly affected soft rot incidence (Fig. 2). There were no soft rot lesions on carrots that had been held in soil saturated for 12 and 24 h at 20 and 25°C or for 12 h at 30°C. After 24 h, incidence continued to increase up to 72 h at 35°C and up to 96 h at all other temperatures. At 96 h, 94% of the carrots had lesions in the 30°C water bath, whereas the incidence was 45% in the 20°C water bath. There was a positive linear relationship between disease incidence and duration of saturation for each temperature. Slopes of the lines for the different temperatures were significantly different ($P = 0.001$).

Comparison of soft rot ability. Bacterial species and temperature significantly affected the amount of carrot tissue consumed by the bacteria. *E. chrysanthemi* caused a greater loss of carrot tissue at higher temperatures than *E. carotovora* subsp. *carotovora* (Fig. 3). Although water-soaked areas were observed on disks inoculated with *E. carotovora* subsp. *carotovora*, neither bacterium reduced carrot disk weight at 15°C. In general, both bacteria caused moderate carrot weight reduction at 20 and 25°C. The greatest weight loss of carrot tissue was caused by *E. chrysanthemi* at 35°C. *E. carotovora* subsp. *carotovora* isolates were relatively less virulent than *E. chrysanthemi* at the higher temperatures. None of the *E. carotovora* subsp. *carotovora* isolates reduced carrot disk weight at 35°C. No weight loss of the noninoculated disks occurred at any temperature.

DISCUSSION

Although *E. chrysanthemi* was reported on carrot in Texas and Taiwan (7,16), this is the first report of *E. chrysanthemi* causing soft rot of carrot in California. In this study, *E. chrysanthemi* caused soft rot craters at temperatures from 20 to 35°C and the development of soft rot increased as

temperature increased. Although *E. carotovora* subsp. *carotovora* is also capable of soft rotting carrots, *E. chrysanthemi* caused soft rot at higher temperatures. Our results are consistent with Lauritzen (9), who found that *E. carotovora* rotted carrots at 24°C but not at higher temperatures. Similar trends have been reported for soft rots on other hosts. Witloof chicory soft rot caused by *E. carotovora* subsp. *carotovora* was severe at 30 but not 35°C (13). Soft rot of *Phyllodendron selloum* caused by *E. chrysanthemi* was most severe at 28 to 34°C (5). *E. chrysanthemi* caused more severe soft rot of potatoes at 30 and 35°C than *E. carotovora* subsp. *carotovora* (8). Although soft rot of carrot caused by *E. carotovora* subsp. *carotovora* occurs consistently at a low incidence in California

fields, it is usually associated with poorly drained areas in a field or near sprinkler system leaks (J. Nunez, *personal observation*). The relatively high soil temperatures in California carrot fields in the late summer of 1998 may explain why *E. chrysanthemi*, and not *E. carotovora* subsp. *carotovora*, resulted in significant economic losses. Experimental temperatures were selected to bracket the soil temperatures recorded at the Shafter Research Station, located in the heart of carrot production in the San Joaquin Valley. Maximum daily soil temperatures, recorded at a depth of 15 cm under irrigated grass, were 23.3 to 31.7°C during August and September 1998. High temperatures and consequent irrigations apparently triggered the carrot soft rot epidemic, in which truckloads of

harvested carrots and several fields were abandoned.

The origin of the inoculum of *E. chrysanthemi* in the carrot fields is not known. Potatoes and onions, which are among the crops grown in rotation with carrots in the San Joaquin Valley, are also hosts and may maintain soil populations of *E. chrysanthemi* (3). *E. chrysanthemi* has previously been isolated from potatoes grown in Kern County (R. M. Davis, *personal observation*). *E. chrysanthemi* may also occur in irrigation water. Although *E. chrysanthemi* was not found in a survey of surface water in North America, *E. carotovora* subsp. *carotovora* was isolated at high frequency (6).

In our experiments, soil saturation was used to ensure a constant film of water surrounding each carrot, which was necessary for disease development. Under California conditions, carrots are typically irrigated by solid set sprinklers every 7 to 10 days for 10 to 12 h. However, irrigation events may be more frequent and for longer duration during very hot or windy conditions. Thus, irrigation may result in a film of water around the carrot root for extended time periods, especially in poorly drained soils. In potato, a film of water surrounding the tuber for 2.5 h at 22°C causes tissues within the tuber to become anaerobic, an important predisposing factor for blackleg development (1,11). Oxygen stress may also be a necessary predisposing factor in soft rot of carrot. This may account for the lack of soft rot in the controls that were inoculated but not saturated with water. Based on these results, growers should limit the length of time carrot roots are surrounded by a film of water, especially at high soil temperatures.

Fig. 2. Influence of temperature and duration of soil saturation on incidence of carrot soft rot. Carrots were grown in 11-cm-diameter styrofoam cups and were 10 to 12 cm long and 1.5 to 2 cm wide at the shoulder when inoculated. Each point represents the average of three replications in each of three trials, four to five carrots per replication. R^2 values for the 20, 25, 30, and 35°C treatments were 0.23, 0.40, 0.66, and 0.55, respectively.

Fig. 3. Reduction of carrot disk weight by three isolates each of *Erwinia chrysanthemi* (E chr) and *E. carotovora* subsp. *carotovora* (Ecc) at five temperatures for 48 h. Controls were sterile water.

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