provided with the combination of fumigant with solarization. Combinations of the above fumigants with napropamide provided consistent control of weeds, nematodes, and soil pathogens and marketable fruit yields similar to that with MBr-Pic.

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A PEER REVIEWED PAPER

FURTHER STUDIES OF BROWN STEM OF CELERY CAUSED BY *PSEUDOMONAS CICHORII*

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Abstract. **Several aspects of brown stem of celery caused by *Pseudomonas cichorii* were studied in a series of greenhouse and growth chamber experiments. Severity of brown stem increased with higher temperatures as measured by the length of characteristic brown streaks in the pith. Average brown streak length was 7.9 cm at 20°C and 29.8 cm at 30°C. Irrespective of which tissue was inoculated, *P. cichorii* moved up and down petioles primarily in the pith, based on observation of symptom patterns and detection of the pathogen with agar prints of petiole cross-sections. Prior inoculation with *P. cichorii* led to reduced development of bacterial soft rot caused by *Erwinia carotovora* subsp. *carotovora*.**

For over 40 years, a firm petiole necrosis, known locally as "brown stem", has plagued Florida celery growers. Outbreaks have been sporadic, but in some years whole plantings have been lost to this malady. The prevailing opinion in the past had been that brown stem was a physiological disorder, related somehow to weather and/or nutritional status of crops. In 1994, we were able to demonstrate that brown stem is caused by petiole infection by *Pseudomonas cichorii* (Pernezny et al., 1994). *P. cichorii* long has been associated with a typical leaf spot disease of celery in southern Florida (Thayer, 1963). Strains isolated from lesions in leaf blades also induce brown stem when inoculated to petioles of test plants. Therefore, brown stem is simply another aspect of the infection of celery by *P. cichorii*. In many ways, brown stem resembled a stem necrosis of chrysanthemum incited by *P. cichorii* (Jones et al., 1983).

Temperature may be important in development of brown stem. Severe outbreaks have been seen at the end of the season (April-May) when temperatures are relatively high. One of the objectives of this work was to determine what effects temperature has on severity of brown stem.

Brown stem is interesting because only cortical and pith tissues become necrotic. Vascular bundles appear as conspicuous green, uninfected areas in cross-section, even when surrounding ground parenchyma is uniformly brown and laden with bacteria. A second objective of this research was to characterize the invasion of specific petiole tissues by *P. cichorii*.

Bacterial soft rot of celery petioles, caused by *Erwinia carotovora* subsp. *carotovora*, is often confused with brown stem in Florida. These two diseases can occur in the same field and may even be manifested on the same plants concurrently. Soft rot can also be a serious post-harvest problem of celery. There is some concern in the industry that an important aspect of brown stem may be to compound soft rot injury. The third objective of this study was to study soft rot development in celery petioles that showed previous brown stem symptoms.

Materials and Methods

Growth of plants. Celery plants of cultivar June Belle or M-9 were grown for 4 mo in 25-cm-diameter plastic pots in an

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air-conditioned greenhouse with a temperature range of 23-27°C. Pots received periodic applications of a slow-release fertilizer (Osmocote 20-20-20, Milpitas, CA). Five to 12 petioles developed per plant, with petiole widths of about 2.5 cm at the time of inoculation. Plants were treated with acephate to control aphids when needed.

Inoculum preparation. Stock cultures of bacteria were maintained in 15% aqueous glycerol at -70°C (Sleesman and Leben, 1978). Working cultures were kept up to 1 mo on slants of King's medium B (King et al., 1954) at 4°C. For individual experiments, plates of King's B were streaked with *P. cichorii*, strain B3 (Pernezny et al., 1994) and incubated at 28°C for 48 hr.

Effect of temperature on disease development. The intent of this study was to compare brown stem development at regimes representing conditions encountered during daylight hours in different celery seasons. A sterile toothpick method was used in these tests. A small amount of bacterial growth was deposited on the tip of a sterile, wooden toothpick, which was then inserted 2 mm into the first node of a test petiole. Each plant was immediately enclosed in a clear polyethylene bag. Five inoculated plants were placed in growth chambers at each of three temperatures: 20°, 25°, and 30°C. One control plant (four petioles wounded with a sterile toothpick only) also was placed in each growth chamber. Bags were opened briefly after 24 hr to remove toothpicks. Bags were removed from plants 3 days later.

Petioles were scored for brown stem development 7 days after inoculation. Each treated petiole was sliced open longitudinally, and the total length of the brown streak above and below the inoculation site was measured.

Movement of P. cichorii *in petiole tissues.* The intent of these studies was to trace colonization of petiole tissues by *P. cichorii.* At least 10 petioles on each of 10 mature plants in 10-cm-diameter pots were inoculated in internode areas using a small amount of fresh bacterial growth on the tip of a number 3 insect pin. Inoculations in three specific tissues, the pith, vascular bundles, and cortex were compared (Fig. 1). The pith area, which constitutes the major portion of the celery petiole volume, was inoculated by insertion of the pin into the interior of the petiole. The vascular bundles, located in the characteristic ribs on the exterior surface of the petiole, were inoculated by carefully inserting pins directly into the ribs. Similarly, inoculations of the cortical tissue were made in areas between the ribs. Depth of penetration of the pinhead was approximately 1 mm below the plant surface. Pins were twisted on removal from the inoculation site to help ensure a deposit of bacterial cells in the wound.

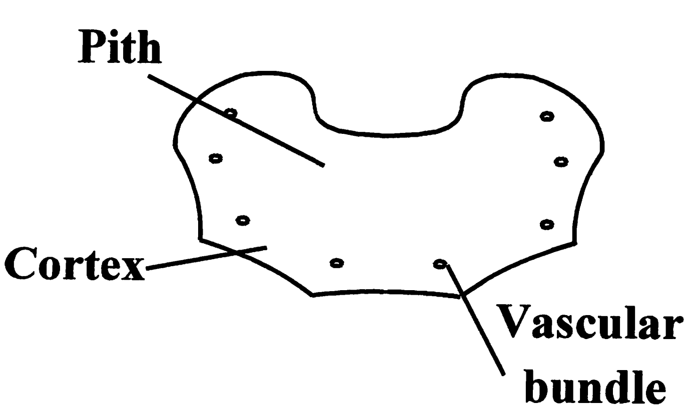

Figure 1. Diagram of cross-section of celery petiole showing various tissues, including pith, cortex, and vascular bundles.

Tissues were examined for colonization by *P. cichorii* at 0, 3, 6, 24, 30, 48 and 72 hr after inoculation. This was done by using a stem printing method similar to that described by Leben (1988). A sterile scalpel was used to cut cross-sections of the petiole. The cuts were initiated from the side of the petiole opposite the inoculation site to minimize the potential for spread of bacteria on the scalpel blade. Cross-sections were sliced at 1-cm intervals above and below the inoculation site from the soil line to the top of the plant and pressed momentarily to the surface of King's medium B plates. When sections were printed, the location of vascular bundles and surrounding tissues was clearly evident by the marks made in the agar. The location of bundles was permanently recorded by placing a small mark with a felt-tip pen on the outside of the bottom of the petri dish opposite the bundles. A total of six petioles was used for destructive samples at each sampling time, representing two petioles inoculated into each type of tissue. Plates were incubated at 28°C for 24-48 hr and examined for growth of *P. cichorii* in cross-section. In a few additional tests, plants were sampled up to 7 days after inoculation.

Development of soft rot in brown stem-infected plants. Celery petioles were inoculated with *P. cichorii* and kept in plastic bags on the greenhouse bench. Bags were removed from three plants (half those originally inoculated). Four petioles on each of these three plants were inoculated 4 days later with *E. c.* subsp. *carotovora.* At the same time, four petioles on three healthy plants were inoculated with *E. c.* subsp. *carotovora.* Plants were bagged for an additional 48 hr after these inoculation procedures. Petioles were removed after 7-13 days, sliced open longitudinally, and observed for symptom development. The extent of soft rot damage above and below the inoculation site was recorded as the total length of affected petiole.

Data analysis. All experiments were repeated two or three times. Data were analyzed by analysis of variance using the Statistical Analysis System (SAS Institute, Inc., Cary, NC, USA) and linear regression using Power Point 97 (Microsoft Corp., Redmond, WA, USA). Means were separated by Student's t-test for two means or Waller-Duncan's method for three or more means.

Results

Effect of temperature on development of brown stem. Brown stem became more severe at the higher temperatures. (Fig. 2). Lengths of the typical brown streaks in the pith tissues of petioles averaged 7.9 cm in plants kept at 20°C, but increased to 29.8 cm for those at 30°C. Brown stem symptoms were not observed in control plants.

Movement of P. cichorii *in petiole tissues.* Irrespective of the placement of inoculum, *P. cichorii* moved primarily in the pith (Fig. 3). Within 6 hr, bacteria introduced into the vascular bundles or the cortex moved laterally in cross-section, often concentrating in pith tissues. After 24 hr, the entire cross-sectional area was colonized. From that point, *P. cichorii* advanced rapidly up and down the petiole, with most movement apparently within the pith. Within 72 hr, pathogen cells were recovered more than 20 cm from the inoculation site both above and below the point of ingress. Most recoveries at locations distant from the inoculation site were from pith tissues, with occasional detection in the cortex. Vascular bundles were virtually free of *P. cichorii.*

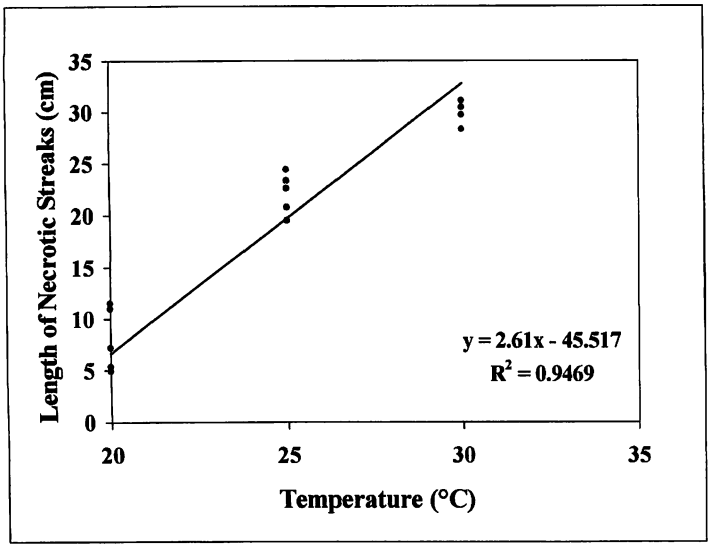

Figure 2. Relationship between temperature and brown stem severity as measured by length of necrotic streaks in petioles of celery plants inoculated at the first node above the soil line.

Development of soft rot in brown stem-infected plants. Previous infection with *P. cichorii* does not predispose celery to soft rot. Soft rot was less severe when petioles were challenged with *P. cichorii* prior to introduction of *E. c.* subsp. *carotovora.* Average length of petiole decay was 30.1 cm when *E. c.* subsp. *carotovora* alone was inoculated into celery compared to 13.2 cm when brown stem-infected petioles were inoculated with soft rot bacteria ($t = 4.13$, $P \leq 0.01$).

Discussion

Brown stem of celery can occur over a range of temperatures. While disease severity increased when infected plants were kept at 30°C, disease development was still substantial at moderate temperatures (25°C). This means that brown stem can be expected throughout the winter growing season in southern Florida. The particularly severe outbreaks noted by the celery industry in late season crops may be explained in part by the positive correlation of disease intensity with temperature. Periods of high humidity and leaf wetness may also contribute to brown stem development as shown for stem necrosis of geranium and chrysanthemum (Jones et al., 1984).

Most plant-pathogenic bacteria that are systemic move within the vascular tissue (Du Plessis, 1984; Pennypacker et al., 1974; Weller and Saettler, 1980; Williams, 1980). In the case of brown stem, *P. cichorii* movement in the petiole is almost exclusively in the pith tissue. This preference for the pith occurs irrespective of the tissue initially inoculated with the bacteria. The mode of movement of *P. cichorii* in the pith is unclear. Celery is mostly water. We thought that the bacterium might move in a transpiration stream that includes the pith as well as the vascular bundles. However, passive movement of water in celery occurs primarily in the vascular bundles, as evidenced by discoloration of only the bundles when freshly cut petioles were immersed in water tinted with food dye (authors, unpublished). Elucidation of the transport mechanism awaits further investigation.

Contrary to a widely held opinion in the industry, brown stem apparently does not predispose celery to bacterial soft rot. Brown stem or bacterial soft rot may occur singly or in combination in celery. With experience, one can accurately differentiate the symptoms of these two diseases even when they occur in the same plant. As shown in this study, the diseases can be keyed to tissue affected. Early bacterial soft rot is confined to the vascular bundles and brown stem is a disease of the pith.

It is difficult at this time to make practical suggestions to producers for management of brown stem. Chemical control is unlikely to be very effective. The bacterium occurs in great numbers in internal tissues, safe from contact sprays of copper and other bactericides. In addition, copper tolerance is widespread among strains of *P. cichorii* recovered from celery farms in Florida (Pohronezny et al., 1994). There does seem to be some varietal differences in susceptibility (Scully et al., 1995). Therefore, future efforts to manage celery brown stem should focus on screening for host-plant resistance.

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