Plant Pathol. J. 19(4) : 205-209 (2003)

Dispersal of Citrus Bacterial Canker Caused by *Xanthomonas axonopodis* pv. *citri* in Nursery Plots of Unshiu Orange

Inn-Shik Myung*, Ki-Woong Nam and Hyeog-Mo Kwon[1]

Plant Pathology Division, National Institute of Agricultural Science and Technology, Suwon 441-707, Korea

[1]Horticultural Division, National Jeju Agricultural Experiment Station, Jeju, Korea

(Received on June 16, 2003; Accepted on August 11, 2003)

Dispersal of citrus bacterial canker caused by *Xanthomonas axonopodis* pv. *citri* on Unshiu orange was investigated in naturally infested nursery plots at Seogwipo in Jeju island, Korea. Based on phage detection, over 2% of the bacterial pathogen overwintered in canker lesions and started to multiply in late May. However, symptoms were first observed 1 month after the phage detection. The disease dispersed non-directionally to nearby plants possibly because of indirect dissemination of the bacterium by rain splashes. The disease increased from late June to late August and decreased thereafter. Population of phage increased constantly, however, disease occurrence somewhat fluctuated due to environmental factors. Disease incidence and severity were correlated with rainfall with wind that occurred 14-32 days earlier from late May to late August.

Keywords **:** Bacterial canker, dispersal, Unshiu orange, *Xanthomonas axonopodis* pv. *citri*

Citrus bacterial canker disease (CBCD or citrus canker) caused by *Xanthomonas axonopodis* pv. *citri* (syn. *X. campestris* pv. *citri*, group A) has been a serious problem in several citrus-producing countries worldwide (Civerolo, 1984). Symptoms of the disease include erumpent and corky lesions on all aerial parts of mature citrus trees such as leaves, stems, and fruits (Schoulties et al., 1987). Fruit spotting caused by the disease reduces marketability, and inter- and intra-country movement of infected citrus fruits has been strictly regulated to preclude entry of the disease into disease-free areas (Koizumi, 1985).

Xanthomonas axonopodis pv. *citri* is known to disperse locally by means of rain splashes. However, its long distance dissemination is directly related to rain with wind when the wind velocity exceeds 8 m/second (Gottward et al., 1988; Serizawa and Inoue, 1975; Serizawa et al., 1969). The bacterium exudes from canker lesions when wet and splashes to nearby plants (Serizawa and Inoue, 1975; Timmer et al., 1991). Bacterial concentrations of 10^5-10^6 colony-forming unit (CFU)/ml have been found in rainwater on diseased foliage. The bacteria have been detected in rainwater at 32 m apart from diseased foliage (Koizumi, 1977).

Rainfall with high wind velocity can cause water soaking which facilitates entry of bacterial cells through stomatal opening into leaves. Inoculum contained in small droplets is carried directionally downwind to neighboring plants during rainstorms with high winds (Gottwald et al., 1988). Gottwald et al. (1989) showed that nursery splash dispersal and establishment of secondary foci to original focus seemed to predominate over wind dissemination of inoculum of *X. axonopodis* pv. *citri*. Citrus canker develops at a temperature range of 5°C to 35°C, with optimum temperature at 30°C (Peltier and Frederick, 1926). Canker lesions are observable with the naked eye about 14 days after infection with *X. axonopodis* pv. *citri*, and the disease usually occurs in immature leaves (Stall and Seymour, 1983).

A phage technique is more rapid and effective than conventional time-consuming procedure in tracing the bacterial population. Phage lysis zones usually become visible within 18-24 hours after spot inoculation. Thus, the technique has been used extensively for studying epidemiology of human pathogens (Anderson and Williams, 1956) and plant pathogenic bacteria (Gross et al., 1991; Liew and Alverez, 1981; Obata, 1974). Previous reports (Myung et al., 2001, 2002) showed that only a single phage group of CP_1 was mainly distributed and corresponded to two bacterial types in Korea.

The purpose of this study was to investigate temporal and spatial dispersal of citrus canker on Unshiu orange from known inoculum sources during the growing season in Jeju, the southern island of Korea.

Materials and Methods

Experimental plots. Eight Unshiu orange trees infected by *X. axonopodis* pv. *citri* were planted at three nurseries designated as A, B, and C in Seogwipo, Jeju island. Each plot consisted of seven

*Corresponding author.
Phone) +82-31-290-0482, FAX) +82-31-290-0453
E-mail) ismyung@rda.go.kr

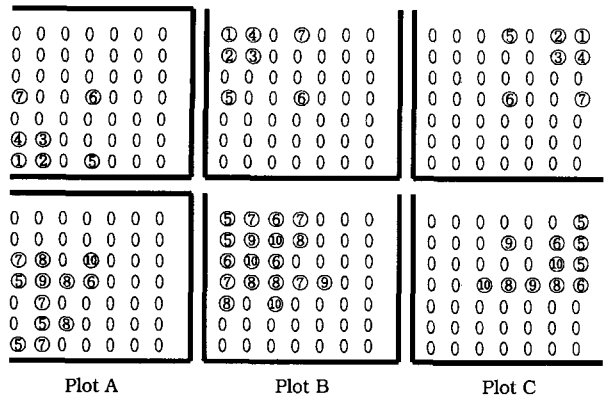

Fig. 1. Diagram of the experimental plots and Unshiu orange trees. (**A**) Plants studied for disease severity and population dynamics. The numbers in circles represent the plants investigated for phages on symptomless leaf surface. Plants infected in a previous season were A1, A3, B1, B2, C1, C2, and C5. Solid lines indicate Japanese Cedars protecting wind. (**B**) Dispersal of citrus cankers was investigated over time. The numbers in circles represent the months the disease occurred on the plants.

rows and seven 13-year-old plants per row. A total of 49 plants per plot were surrounded by Japanese Cedars on two to three sides to protect from sea winds (Fig. 1). Dispersal, incidence, and severity of the disease, and the phage population on leaf surface were investigated. Space between rows was 1.5 m. Meteorological data were obtained from Seogwipo branch of Jeju local station of Meteorology.

Disease incidence and severity. The disease was investigated on March 23, April 23, May 24, June 25, July 31, August 26, September 22, and October 25 in 1993. Disease incidence was estimated by counting plants with at least on canker lesion on leaves in each plot. Disease severity was examined in seven plants per plot as shown in Fig. 1A and disease severity was determined by the number of diseased leaves divided by 200 leaves in each plant (Fig. 1A).

Phage detection from citrus canker lesions. The method for detection of *X. axonopodis* pv. *citri* that over-wintered in canker lesions of Unshiu oranges was described previously by the authors (Myung et al., 2001). Ten canker lesions obtained from diseased leaves were macerated with 1 ml of sterile distilled water in a sterile mortar and pestle. Twenty μl of the supernatant obtained by centrifugation was dropped on agar with BC 1 of *X. axonopodis* pv. *citri* isolated from Unshiu orange. The plates were incubated at 25°C for 24 hours to detect phages. Ten putative phage solutions were prepared every sampling day.

Population of phage on leaf surface. In order to understand the bacterial dissemination, population of phages on symptomless leaf surface of seven plants per plot was assayed at the sampling days described above. Symptomless leaves randomly detached were cut into small pieces with scissors then washed in 50-ml sterile distilled water in a 250-ml flask at 4°C and centrifuged at 250 rpm for 30 minutes. Three milliliter of the suspension was mixed with 7 ml of peptone sucrose soft agar medium (Bacto peptone 10 g, sucrose 10 g, sodium glutamate 1 g, Bacto agar 7 g, distilled water 1 L) containing BC 1 strain of *X. axonopodis* pv. *citri* and poured into a plate. Phage number on symptomless leaf surfaces was determined after 24-hour incubation at 28°C.

Results

Disease incidence and disease dispersal. Phages were first detected on May 24 in two out of ten phage solutions. However, the disease was first observed about one month after the phage detection in B and C plots and increased gradually thereafter (Fig. 2). The disease incidence on March 23 was about 0.05% in the plots, but increased gradually to 0.22, 0.34, and 0.22% in plots A, B, and C, respectively. The disease spread to new healthy leaves throughout the growing season of this year.

The disease dispersed non-directionally to other parts of the diseased plant and to neighboring plants. The increase in disease occurrence seemed to be largely affected by rain since it abruptly increased 14-32 days after rainfall with

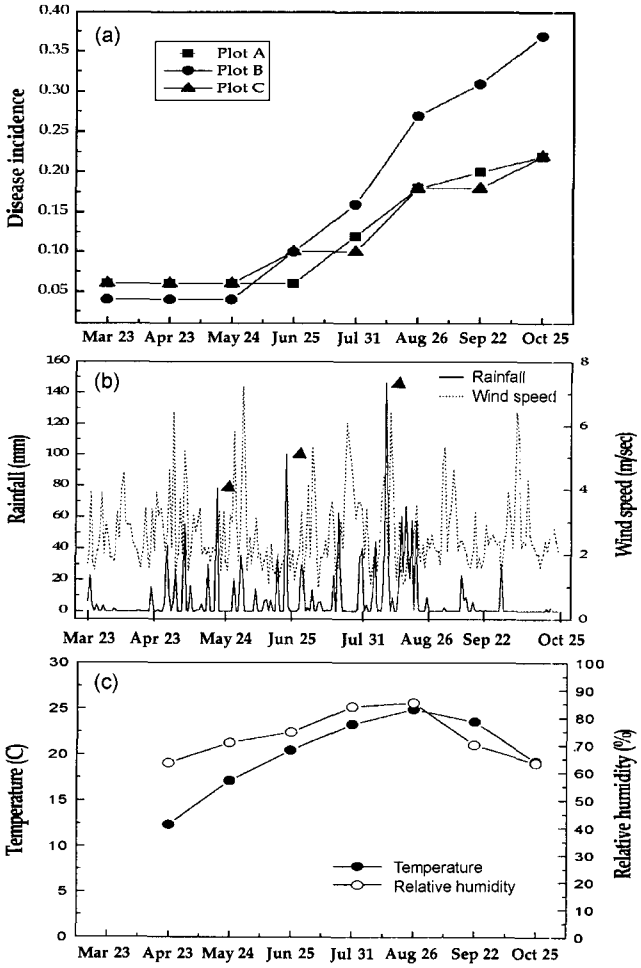

Fig. 2. The development of the bacterial canker on Unshiu orange was compared with rainfall, wind speed, temperature, and relative humidity.

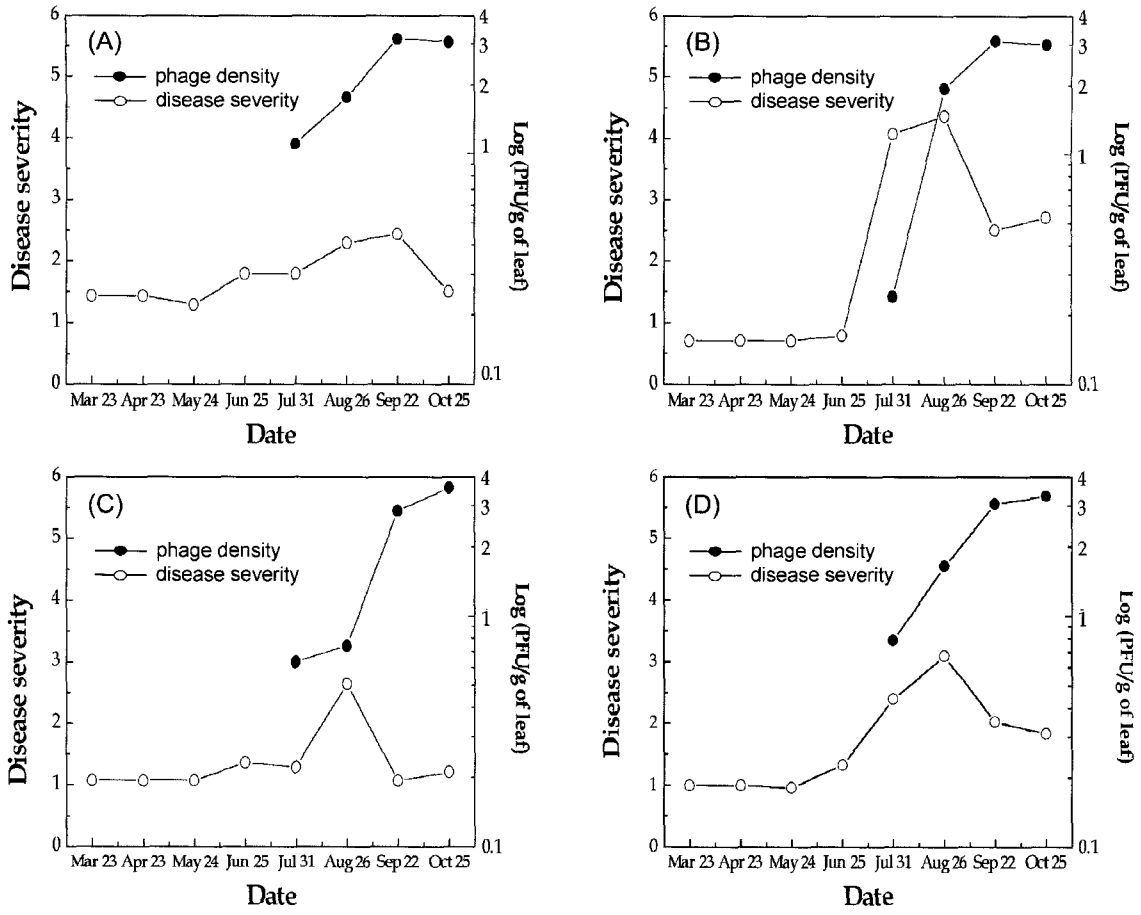

Fig. 3. (**A-C**) Disease severity and population dynamics of symptomless leaf surface phage for seven plants indicated in Fig. 1A. (**D**) Average disease severity and the dynamics of symptomless leaf surface.

strong winds (Fig. 2). However, it also increased gradually during the moderate rainy season from September to October. Wind speed exceeding 3 m/seconds that affect disease spread occurred over six times each month throughout the growing season (Fig. 3). Monthly average temperature from late June to late October was over 18°C and the disease occurred on new leaves during that time.

Disease severity and population dynamics of symptomless leaf surface phage. Disease severity of the inoculum plants on March 23, 1993 was estimated at 1.4, 0.6, and 1% in plots A, B, and C, respectively. On October 25, the disease severity increased to 1.5, 2.8, and 1.2% in plots A, B, and C, respectively. The disease severity in all plots reached a peak in August in plots B and C, and in September in plot A, but decreased thereafter (Fig. 3). The disease severity was also largely dependant on rainfall with wind. It sharply increased 14-32 days after rainfall with wind.

Phages on symptomless leaves were detected at 30 days after the disease first occurred and their population increased abruptly (Fig. 3). The phage population declined in October in plots A and C. The number of phages was closely related to disease incidence and disease severity.

Discussion

It was shown in this study that the bacterial pathogens overwintered in canker lesions could be detected using phage technique, which might have served as a primary inoculum source for the disease. Detection of bacterial pathogens in the lesions in spring season is important to warn dispersal of the pathogens to healthy plants. The disease increased slowly during the first season but increased sharply from the next season. The bacterial population in lesions which formed at spring flush remained consistent throughout the growing season (Stall and Seymour, 1983). In general, the growth of Unshiu orange in Jeju island flushes three times at spring after winter dormancy, summer, and autumn. Thus, it was presumed that rapid increase of canker disease from June 23 to October 23 could be secondary and/or third infection from the lesions produced in spring and summer.

Gottwald et al. (1992) suggested that citrus bacterial spot

(CBS) and citrus canker may have similar wind speed threshold of more than 8 m/second to cause infection by inoculum carried by windblown rain. Rainfall with high wind velocity could disseminate bacterial pathogens to nearby plants, and cause water soaking or wounds on plant parts to facilitate entry of bacterial cells through stomatal opening or wounds into leaves. Gottwald et al. (1992) found that disease development of CBS was detected as far as 4.1 m from nursery edge, even where wind speed was only about 5 m/sec. Disease development with wind speed exceeding 8 m/second was not recorded. However, three rainfalls with wind speed exceeding 4 m/sec were recorded 14-32 days earlier before the disease became severe.

The disease dispersed non-directionally to form a cluster of about 7.5 m in diameter (Fig. 1B). Exudation of *X. axonopodis* pv. *citri* from canker lesions occurs when water is added to wells surrounding young lesions (Timmer et al., 1991). Rainfall is a major contributing factor to splash dispersal of the bacterial pathogens from canker to nearby plants (Gottwald et al., 1988, 1989 and 1992). Danos et al. (1984) found that high concentrations of the bacterial pathogens in rainwater were detected from foliages infected with CBCD and under diseased plants. Rainfalls in growing season can promote local dispersal of the bacterial pathogens from canker lesions to nearby plants (Serizawa and Inoue, 1975; Serizawa et al., 1969). Raindrops hitting a canker lesion oozing with bacterial pathogens would fragment into smaller droplets, which usually would reach the neighboring plants in the lattice. Gottwald et al. (1988) suggested that these droplets could be carried downwind to nearby plants during rainstorms with high winds. However, splash dispersal of inoculum to nearby plants was more probable, because the close spacing between plants and the Japanese Cedars protecting them from the wind could mask directional spread due to wind dissemination. Thus, the diseased plants would coalesce to form a cluster, eventually serving as the foci for dispersal of *X. axonopodis* pv. *citri* for the next season.

Phages on symptomless leaf surfaces were not detected until late June when the disease dispersal first occurred. This is likely due to the short-live nature of *X. axonopodis* pv. *citri* on leaf surface, and/or that the levels of surface phages were below the detection threshold of 0.25 of $\log_{10}$ (PFU/g) by the surface washing assay. Timmer et al. (1991) showed that *X. axonopodis* pv. *citrumello* on leaves of Swingle citrumello was alive only for a short period of time. Gottwald et al. (1992) explained that leaf surface bacteria in CBS-*X. axonopodis* pv. *citrumello* pathosystem may not necessarily contribute directly to disease development but can exist as causal leaf surface inhabitants.

In this study, population dynamics of phages on symptomless leaf surfaces were found to be related to disease severity 30 days after disease dispersal. The increase in phage population indicated that bacterial pathogens were disseminated from canker lesions to healthy leaves. Since new leaves of Unshiu orange grow continually during the cultivation season, the disease severity also gradually increases throughout the season.

However, the disease severity declined from 26 August to October 25 despite constant increase in phage populations (Fig. 3). The disease reduction might be due to defoliation of the diseased leaves by environmental factors such as winds from early September to October.

Temperatures for citrus canker development ranged from 5°C to 35°C and was optimum at about 30°C (Petlier and Frederick, 1926). However, disease development on Unshiu orange in this study under relatively low temperature in autumn could be due to young leaves flushed during that season. Also, the susceptibility of Unshiu orange to *X. axonopodis* pv. *citri* contributed to the disease development.

References

Anderson, E. S. and Williams, R. E. O. 1956. Bacteriophage typing of enteric pathogens and staphylococci and its use in epidemiology. *J. Clin. Pathol.* 9:994-127.

Civerolo, E. L. 1984. Bacterial canker disease of citrus. *J. Rio Grande Val. Hortic. Soc.* 37:127-146.

Danos, E., Berger, R. D. and Stall, R. E. 1984. Temperal and spatial spread of citrus canker within groves. *Phytopathology* 74:904-908.

Gottwald, T. R., Graham, J. H. and Richie, S. M. 1992. Relationship of leaf surface populations of strains of *Xanthomonas campestris* pv. *citrumello* to development of citrus bacterial spot and persistence of disease symptoms. *Phytoplathology* 82:625-632.

Gottwald, T. R., McGuire, R. G. and Garran, S. 1988. Asiatic citrus canker: spatial and temporal spread in simulated new planting situations in Argentina. *Phytopathology* 78:739-745.

Gottwald, T. M., Timmer, L. W. and McGuire, R. W. 1989. Analysis of disease progress of citrus canker in nurseries in Argentina. *Phytopathology* 79:1276-1283.

Gross, D. C., Powelson, M. L., Regner, K. M. and Radamaker, G. K. 1991. A bacteriophage-typing system for surveying the diversity and distribution of strains of *Erwinia carotovora* subsp. *carotovora* in potato fields. *Phytopathology* 81:220-226.

Koizumi, M. 1977. Relation of temperature to the development of citrus canker in the spring. *Proc. Int. Soc. Citric.* 3:924-928.

Koizumi, M. 1985. Citrus canker: the world situation. In: Citrus Canker. An International Perspective. L. W. Timmer, ed. *Proc. Symp. Intst. Food Agric. Sci., University of Florida.* pp. 2-7.

Kuhara, S. 1978. Present epidemic status and control of the citrus canker disease, *Xanthomonas citri* (Hasse) Dow., in Japan. *Rev. Plant Prot. Res.* 11:132-142.

Liew, K. W. and Alverez, A. M. 1981. Phage typing and lysotype

distribution of *Xanthomonas campestris*. *Phytopathology* 71: 274-276.

Myung, I.-S., Cho, Y., Lee, Y.-H. and Kwon, H.-M. 2001. Phage typing and lysotype distribution of *Xanthomonas axonopodis* pv. *citri*, the causal agent of citrus bacterial canker in Korea. *Plant Pathol. J.* 17:336-341.

Myung, I.-S., Nam, K.-W. and Cho, Y. 2002. Cultural characteristics of *Xanthomonas axonopodis* pv. *citri* bacteriophages CP1 from Korea. *Plant Pathol. J.* 18:333-337.

Obata, T. 1974. Distribution of *Xanthomonas citri* strains in relation to the sensitivity to phages of Cp1 and Cp2. *Ann. Phytopath. Soc. Jpn.* 40:6-13.

Peltier, G. L. and Frederick, W. J. 1926. Effects of weather on the world distribution and prevalence of citrus canker and citrus scab. *J. Agric. Res.* 32:147-164.

Schoulties, C. L., Civerolo, E. L., Miller, J. W., Stall, R. E., Krass, C. J., Poe, S. R. and DuCharme, E. P. 1987. Citrus canker in Florida. *Plant Dis.* 71:388-395.

Serizawa, S. and Inoue, K. 1975. Studies on citrus canker. III. The influence of wind blowing on infection. *Bull. Schizuoka Pref. Citrus Exp. Stn.* 11:54-67.

Serizawa, S., Inoue, K. and Goto, M. 1969. Studies on citrus canker. I. Dispersal of the citrus canker organism. *Bull. Schizuoka Pref. Citrus Exp. Stn.* 8:81-85.

Stall, R. E. and Seymour C. P. 1983. Canker, a threat to citrus in the gulf-coast states. *Plant Dis.* 67:581-585.

Timmer, L. W., Gottwald, T. R. and Zitko, S. E. 1991. Bacterial exudation from lesions of Asiatic citrus canker and citrus bacterial spot. *Plant Dis.* 75:192-195.