

## Citrus Canker: Developments down the lane

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### ABSTRACT

*Citrus canker (caused by Xanthomonas citri ssp. citri, a gram negative bacterium) is considered one of the most feared bacterial diseases of limes/lemons, though affects all types of citrus species. However, there is hardly any citrus species immune to citrus canker in field having severe incidence. Number of field screenings against citrus canker showed Calamondin and Kumquats are highly resistant against citrus canker, while mandarins like Ponkan, Satsuma, Tankan, Cleopatra, Sunki, Sun Chu Sha etc are, though, reported resistant to citrus canker, but their performance under severe incidence of canker still remains a question mark. Chronologically not much development have taken place, except the physical eradication through pruning and spray of antibiotics and copper-based fungicide still hold the anticipated promise. But of late, biological control of canker using microbial antagonists along with obligate endophytes have shown better results under field conditions. These developmental efforts opened up the renewed role of endophytes and novel bioagents to be attempted in a big way against citrus canker. All these issues have been briefly highlighted in this review.*

**Key words:** Citruscanker, pathogen, tolerance, field screening, antagonists, endophytes

### INTRODUCTION

Citrus is grown in tropical and subtropical regions of the world and occupies a wide range of latitude over which it is being cultivated (Srivastava, 2014). It occupies an important place in the wealth and economy of India as third largest fruit industry after mango and banana (Srivastava *et al.*, 2015). In India, citrus is grown in an area of 1,024 thousand hectares with a total production of 11,581 thousand MT (NHB, 2019). Citrus is threatened by a number of pests and diseases, out of which citrus canker is one of the major diseases, particularly in limes/lemons in all citrus growing states of India (Das, 2003). The disease causes an extensive damage to citrus and severity of this infection varies with different species/varieties and the prevailing climatic conditions. The disease is reported in Pakistan, India, China, Japan, south-east Asia and the other islands of Indian Ocean (Das, 2003). In India, citrus canker was first reported by Luthra and Satter (1942) from Punjab and now known to be prevalent in almost all the states (Nirvan, 1961).

### CITRUS CANKER

Citrus canker caused by gram negative bacterial pathogen, *Xanthomonas citri* ssp. *citri* of Xanthomonadaceae family is one of the

most feared citrus diseases, affecting virtually all types of citrus crops (Pitino *et al.*, 2015). It causes an extensive damage to citrus crops where infected trees display unsightly lesions to form on leaves, fruit and stems. When citrus infection occurs in the early growing stage, the fruits crack or become malformed as they grow, and the heavily infected ones fall prematurely. Light infection in later growth stage may cause only scattered canker lesions on the surface of fruits but makes fresh fruits unacceptable for market. Severe infection results in defoliation, deformation of fruit and premature fruit drop (Chand and Pal, 1982).

The causative pathogenic bacterium was first isolated by Hasse in 1915 from diseased samples of Florida, Texas and Mississippi and he named it *Pseudomonas citri* following its characterization and pathogenicity in citrus (Hasse, 1915). Subsequently it was placed under different genera by different workers and in 1939 it was named as *Xanthomonas citri* (Doidge, 1916; Dowson, 1939). *X. citri* was divided into different strains where strain A was assigned to those associated with Asiatic citrus canker, strains C to those causing canker only in Key lime (*Citrus aurantifolia*), and B to strains with wider host range (Namekata and Oliveira, 1972; Rosetti, 1977). In 1978 the bacterium was again placed in *X. campestris* pv. *citri* to preserve *citri* at the

infrasubspecific level (Young *et al.*, 1978). With the advent of molecular techniques study on Citrus canker pathogen got a new momentum. Using DNA–DNA hybridization (DDH) Vauterin *et al.* (1995) transferred the A strains to *X. axonopodis* pv. *citri* whereas the B and C strains were accommodated under *X. axonopodis* pv. *aurantifolii*. Recently, Constantin *et al.* (2016) by using a polyphasic approach have proposed significant changes to xanthomonad taxonomy, including the *X. citri*. For now, *Xanthomonas citri* subsp. *citri* (*Xcc*) is used for bacterium causing Asiatic citrus canker disease (Luo *et al.*, 2020). Das (2003) reported the bacterium *Xcc* is rod-shaped measuring 1.5-2.0 × 0.5-0.75 µm, gram-negative and has a single polar flagellum with obligate nature. Colonies on culture media are usually yellow due to the production of xanthomonadin pigment.

The geographical origin of citrus canker is a matter of controversy. Lee (1918) earlier reported the origin of disease in southern China, and assumed *Fortunella hindsii* to be the wild host plant. Earlier, Citrus canker was first introduced to Florida around 1910 on trifoliate citrus rootstock imported from Japan, and was first discovered in a Monticello, Florida nursery in 1912. This disease outbreak contributed in part to passage of the Plant Quarantine Act of 1912 and the Florida Plant Act of 1915. The last infected tree was removed from Florida in 1927, and eradication of the disease from the state was officially declared in 1933 (Mani Skaria and de Graca, 2012). Later, Fawcett and Jenkins (1933) reported that citrus canker originated in India and Java, rather due to detection of canker lesions on the oldest citrus herbaria kept at the Royal Botanical Gardens in Kew, England (*Citrus medica* collected from India during 1827-1831 and *C. aurantifolia* from Indonesia during 1842-1844). These findings suggested the origin of disease in the tropical areas of Asia, such as south China, Indonesia, and India, where *Citrus* species are presumed to have originated and to have been distributed to other citrus-growing areas in the form of budwood. Citrus canker was described afterwards in the Gulf States regions of USA in 1915. The Gulf States outbreak is believed to have resulted from a shipment of infected nursery stock from Asia (Dopson, 1964). The disease also appeared earlier this century in South Africa (Doidge, 1916) and Australia (Garnsey *et al.*, 1979).

## Distribution and economic importance

In spite of the heightened regulations imposed by many countries to prevent introduction, the disease continues to increase through different geographic ranges. Citrus canker presently occurs in over thirty countries in Asia, the Pacific and Indian Ocean islands, South America and the Southeastern USA (Sharma and Sharma, 2009). Worldwide, millions of dollars are spent annually on prevention, quarantines, eradication programs, and disease control. Undoubtedly, the most serious consequence of citrus canker infestation is the impact on commerce resulting from restrictions to interstate and international transport and sale of fruit originating from infested areas (Graham *et al.*, 2008).

## Disease cycle and epidemiology

The pathogen of citrus canker, *Xcc* survives in naturally occurring lesions. Cankerous leaves, twigs and branches constitute the main source of inoculum. Since affected leaves drop early, they may not serve as the main source of inoculum (Nirvan, 1963). Rao and Hingorani (1963) observed that the bacterium survives up to 6 months in the infected leaves. The pathogen can survive in diseased twigs up to 76 months (Chakravarti *et al.*, 1966). Earlier Vasudeva (1958) observed that under desiccation at 30°C, the pathogen survived for 11 or 12 days, while Paracer (1961) observed the bacterium resistant to drying and killed after 120 days under laboratory temperature. Lesion development and bacterial multiplication may be directly related to host resistance (Koizumi, 1979). Presence of free moisture on the host surface for 20 min. has been observed conducive for successful infection (Ramakrishnan, 1954).

Canker develops more severely on the side of the tree exposed to wind-driven rain. Rainwater collected from foliage with lesions contained bacterial population between 10<sup>5</sup>-10<sup>8</sup> cfu/ml (Stall *et al.*, 1980). If the average wind speed during rains exceeds 8m/sec (18mph), the disease may be severe (Kuhara, 1978). Spread over longer distances, upto 7 miles can occur during severe tropical storms, hurricanes and tornadoes (Gottwald *et al.*, 2001). There is no record of seed transmission. Nursery workers

can carry bacteria from one nursery to another on hands, cloths and equipments. Similarly, spread can also result from movement of contaminated budwood or contaminated budding equipments. Environmental condition plays a crucial role in the epidemic development of citrus canker disease. Temperature between 20-30°C with evenly distributed rainfall is suitable for serious disease development (Reddy, 1984). The incidence of canker has been observed to have strong relation with leaf miner. The Asian leafminer, *Phyllocnistis citrella* Stainton, can infest leaves, stems, and fruit and greatly increase the number of individual lesions which quickly coalesce and form large irregular shaped lesions that follow the outlines of the feeding galleries. Trees with wounds caused by leaf miner remain susceptible for 7-14 days compared to only 24 hours for wounds caused by wind, thorns or pruning (Filho and Hughes, 2000). However, there are no published data that the leaf miner serves as a true vector of canker inoculum.

### Disease diagnosis

Citrus canker disease is diagnosed most of the times using canker lesions on fruit peels. However, rapid development of molecular methods for characterization of bacteria over the past decade has greatly simplified and improved the detection and identification of plant pathogenic bacteria (Alvarez, 2004; Lopez *et al.*, 2005). Several sets of primers have been designed for polymerase chain reaction (PCR) detection of *Xcc*. Among them, some are based on sequences from plasmid borne genes (Cubero and Graham, 2005), rDNA sequences (Cubero and Graham, 2002) and general or pathogenicity regulatory factors (Coletta-Filho *et al.*, 2006). An integrated approach for reliable detection of *Xcc* in lesions of fruit samples, employing several techniques and with real-time PCR using a TaqMan probe as the fastest and most sensitive screening method, has been established and validated and is proposed as a useful tool for the analysis of *Xcc* on fresh fruits (Golmohammadi *et al.*, 2007).

### Disease management

Citrus canker pathogen was eradicated from Australia, New Zealand, South Africa, and the United States during the first half of the 20th

century, but was not eradicated in Brazil and Argentina in more recent times. The geographical range of citrus canker continues to enlarge; the bacterium was introduced into Mexico and Yemen Arab Republic during the past 5 years, and discovery of the bacterium in Florida in 1984 prompted another eradication campaign in the United States to manage the disease (Stall *et al.*, 1987). Chronologically, the most effective management of canker is by supplementing the use of resistant cultivars with integrated systems of compatible cultural practices and phytosanitary measures, including quarantine and regulatory programmes. Thereafter, the basic strategies of the specific methods are to avoid, exclude, or eradicate the pathogen, to reduce the amount of inoculum available for infection, to minimize dissemination of the pathogen, and to protect susceptible tissue from infection (Civerolo, 1981). However, the recent approaches including chemical, bio-intensive approaches especially the microbial bioagents have opened up new avenue for successful management of the disease.

### Host Resistance

Citrus plants vary in their susceptibility to canker. Grapefruit and Mexican limes are highly susceptible. Sweet oranges and lemons are moderately susceptible. Mandarins are moderately resistant. Identification of resistant plants is one of the most effective methods of disease management; the ability to screen for resistant seedlings plays a key role in the production of a long-term solution to canker. Colonies of the causal bacterium, isolated from leaf, stem, and fruit lesions, appeared similar to the Asiatic group of strains on the nutrient agar plate, but the growth on lima bean agar slants was less mucoid. The bacterium produced erumpent, pustule-like lesions of typical Asiatic citrus canker syndrome after inoculation into Key/Mexican lime, but brownish, flat, and necrotic lesions on the leaves of Duncan grapefruit, Madame Vinous sweet orange, sour orange (*C. aurantium*), citron (*C. medica*) and trifoliolate orange (*Poncirus trifoliata*) according to studies carried out by Sun *et al.* (2004). Pitino *et al.* (2015) have developed a rapid method of screening for citrus canker resistance. Incubating citrus seedlings with a harmless,

canker-derived peptide molecule triggers plant defenses including the release of reactive oxygen species. The quantity of reactive oxygen species produced, which can easily be measured by leaf.

Table 1: Relative susceptibility/resistance to citrus canker of commercial citrus cultivars and species (Based on hypersensitivity test using challenge inoculation test, not representing the true sensitivity tests and field evaluation studies)

Citrus canker rating	Description of commercial citrus cultivars/species
Highly resistant	Calamondin ( <i>C. mitis</i> ), Kumquats ( <i>Fortunella</i> spp.)
Resistant	Mandarins ( <i>C. reticulata</i> Blanco) : Ponkan, Tankan, Satsuma, Cleopatra, Sunki, Sun Chu Sha Mandarins: Tangerines, Tangors, Tangelos ( <i>C. reticulata</i> hybrids); Cravo, Dancy, Emperor, Fallgelo Fairchild, Fremont, Clementina, Kara, King Lee, Murcott, Nova, Minneola, Osceola, Ortanique, Page, Robinson, Sunburst, Temple, Umatilla, Willowleaf (all selections), Nagpur mandarin, Khasi mandarin, Kinnow mandarin, Sweet oranges ( <i>C. sinensis</i> ) -- Berna, Cadenera, Coco, Folha Murcha, IAPAR 73, Jaffa, Moro, Lima, Midsweet, Sunstar, Gardner, Natal, Navelina, Pera, Ruby Blood, Sanguinello, Salustiana, Shamouti, Temprana and Valencia, Mosambi, Sathgudi, Malta
Less susceptible	Acid lime ( Pramalini, Vikram, Chakradhar, Ganadevi, Sai Sharbati, Sharbati, Elaichi nimboo ) Lemons : Lisbon, Eukeka, Assam, Kachai Sweet oranges: Hamlin, Marrs, Navels (all selections), Parson Brown, Pineapple, Piralima, Ruby, Seleta Vermelha (Earlygold), Tarocco, Westin.
Susceptible	Mandarins: Tangerines, Tangelos -- Clementine, Orlando, Natsudaidai, Pummelo ( <i>C. grandis</i> ). Limes ( <i>C. latifolia</i> ) : Tahiti lime. Trifoliate orange ( <i>Poncirus trifoliata</i> ) : Citranges/Citrumelos ( <i>P. trifoliata</i> hybrids)
Highly susceptible	Grapefruit ( <i>C. paradisi</i> ); Mexican/Key lime ( <i>C. aurantiifolia</i> ); Lemons ( <i>C. limon</i> ); and Pointed leaf Hystrix ( <i>C. hystrix</i> )

Source: Zubrzycki and Zubrzycki (1981), Leite and Mohan (1984), Gottwald et al. (1993), Graham et al. (2001), Singh and Srivastava (2019)

Luminescence accurately reflects the level of resistance against canker. Applying this simple test to large numbers of citrus seedlings should enable rapid identification of strains with potential for breeding resistant citrus crops. The suggestive list featuring different citrus species for citrus canker, though showed some species as canker resistant (Table 1), but their field efficacy under severe incidence is yet to be ascertained.

### Mechanical and chemical approaches

Eradication measure of citrus canker under endemic condition is considered not feasible. Canker incidence under these conditions can be reduced considerably by taking integrated management approach consisting of: i. using canker-free nursery stocks, ii. pruning all the infected twigs before monsoon and burning them, iii. Periodical spraying of suitable copper-based bactericides (to reduce inoculum build-up on new flushes and to protect

expanding fruit surfaces from infection) along with an insecticide (to control insect injury), iv. taking some precautions to reduce the risk of spread of disease in orchards and nurseries and v. by evolving canker-resistant varieties suited to local environmental conditions (Das and Singh, 2001). Krishun and Chand (1987) recommended two pruning along with 4 sprays of 5000 ppm copper oxychloride or 1% Bordeaux mixture is reported to be effective against the disease. Later, studies advocated the treatment of 500-1000 ppm streptomycin sulphate with 1% glycerine on acid lime (Rangaswami et al., 1959), agrimycin (Sawant et al., 1985), streptomycin (Mathur et al., 1973) and streptomycin in combination with Bordeaux mixture (Krishna and Nema, 1983). Integrated application of pruning of infected twigs copper oxychloride (0.3%), streptomycin (100 ppm) and neem cake suspension was observed very effective in controlling the disease (Das and Singh, 2000). These efforts, however, proved only partially effective.

## Biological management

Plant origin botanicals as an alternate to synthetic pesticides have proved to be effective chemotherapeutants and can be used as valuable source of natural pesticides (Balandrin *et al.*, 1985; Mahajan and Das, 2003; Khan *et al.*, 2018; Bora and Bora, 2008a; 2009). Biological control agents, including antagonistic foliar bacteria, and plant growth-promoting bacteria, and bacteriophages have been successfully used in several pathosystems (Balogh *et al.*, 2003) and are also promising candidates for control of asiatic citrus canker. Bacteriophages have been used effectively for controlling several diseases caused by species of *Xanthomonas*, including bacterial spot of peach, caused by *X. compestris* sp. *pruni*. (Obradovic *et al.*, 2005), geranium bacterial blight, *X. compestris* sp. *pelargoni* (Flaherty *et al.*, 2000) etc. The prevention of the deleterious effects of phytopathogenic organisms is achieved by production of wide range of antibacterial and antifungal compounds conferring its wide use as biocontrol agents (Hayat *et al.*, 2010). A more sustainable alternative to combat these diseases is the control of *Xanthomonas* by microorganisms directly or indirectly through the use of its secondary metabolites involved in biocontrol (Marin *et al.*, 2019).

Fungal bioagents inhibit pathogenic invasion through phenomena of mycoparasitism, antibiosis and competition (Bora *et al.*, 2013). The inhibition by the bio-agent *Trichoderma* spp. is due to the volatile and non-volatile metabolites and cell wall degrading enzymes. Certain strains of plant growth promoting *Pseudomonas* sp., have been already used as biocontrol agents to suppress citrus canker, and offer an attractive way to improve crop growth and development, replacing or supplementing chemical pesticides (Pandaya, 2015). Some strains of bacteria *viz.*, *Pseudomonas syringae*, *Erwinia herbicola*, *Bacillus subtilis* and *Pseudomonas fluorescens* isolated from citrus phylloplane were reported to be antagonistic *in vitro* to the canker pathogen (Kalita *et al.*, 1996; Unnimalai and Gnanamanickam, 1984). However, it seems difficult to find antagonistic bacteria that reside stably on smooth surfaces of mature citrus leaves.

Das *et al.* (2014) studied the inhibitory strain of *Bacillus subtilis* (S-12) using single

spray of aqueous suspension ( $2.7 \times 10^9$  cells/ml) of bacterial cells spread on 5 batches (6 numbers of plants/batch) of plants keeping 4 batches unsprayed. A sharp decline of the disease was recorded at 20 days after treatment indicating that the spore forming bacteria might have taken over on the leaf surface of the plants. Kalita *et al.* (1996) reported that, among the tested species of bacteria *viz.*, *Bacillus subtilis*, *B. polymyxa*, *Pseudomonas fluorescens*, and three species of fungi *viz.*, *Aspergillus terreus*, *Trichoderma viride* and *T. harzianum* isolated from the phylloplane of citrus variety Assam lemon (*Citrus limon*) inhibited the growth of *Xcc in vitro*. However, *Bacillus subtilis* was found to be most effective antagonist exhibiting maximum (14.7mm) inhibition of the pathogen and reducing the disease incidence to an extent of 61.19 percent. *Pseudomonas* species showed traits of effective biological control agent (BCAs) against several phytopathogens (Bora *et al.*, 2016a; 2016b; 2019). The *Pseudomonas* strains most usually recognized for their biocontrol activity against both eukaryotic and prokaryotic phytopathogenic microorganisms are *P. fluorescens*, *P. protegens*, *P. chlororaphis* and *P. putida* (Ramette *et al.*, 2011; Sharma *et al.*, 2020). *Pseudomonas* strains isolated and identified from soil samples added with compost (Bora and Bora, 2008b; Bora *et al.*, 2013) displayed a strong activity against *Xcc* due to the production of secondary metabolites by the *Pseudomonas* strains isolated. Daungfu *et al.* (2019) isolated numbers of endophytic bacteria from *C. aurantifolia*, *C. hystrix*, *C. maxima*, *C. nobilis*, *C. reticulata* and *C. sinensis* were 28, 25, 29, 42, 12 and 34 isolates, respectively. The selected endophytic bacteria that were effective against *X. citripv. citriwere Bacillus amyloliquefaciens* LE109, *B. subtilis* LE24 and *B. tequilensis* PO80. These studies showed that endophytic bacteria could play an important role in citrus canker control.

## FUTURISTIC VIEWS

The futuristic headways with regard to diagnosis and management of citrus canker lies in developing large scale rapid field screening methodology, disease forecasting models considering the hot spots of limes production across the world and developing consortium of microbial antagonists using bacterial endophytes

to name few important ones. The citrus species which are considered resistant to citrus canker

need to be tested under severe incidence of canker load.

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