Citrus canker – A review

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Abstract

Of all the agricultural pests and diseases that threaten citrus crops, citrus canker is one of the most devastating. The disease, caused by the bacterium *Xanthomonas axonopodis* pv. *citri,* occurs in large areas of the world's citrus growing countries including India. At least 3 distinct forms or types of citrus canker are recognized. Among these, Asiatic form (Canker A) is the most destructive and affects most of the major citrus cultivars. Severe infection of the disease produces a variety of effects including defoliation, dieback, severely blemished fruit, reduced fruit quality and premature fruit drop. Warm, humid, cloudy climate, along with heavy rainfall and strong wind promotes the disease. Control of canker in countries or regions where the disease is not present include quarantine or regulatory programme to prohibit introduction of infected citrus plant material and fruit, as well as continuous and strict surveying in the field and the immediate destruction of infected trees. In countries where canker is present, integrated systems of compatible cultural practices and phytosanitary measures consisting of resistant hosts, removal of inoculum sources, properly designed windbreak systems, timely application of protective copper-containing and/or antibiotic sprays are generally the most effective means of disease management. This paper reviews the current state of knowledge and understanding on pathogens and strains associated with the disease and their identification, host-pathogen interaction, molecular mechanism of pathogenicity, epidemiological aspects and management practices.

Key words : Citrus canker, *Xanthomonas axonopodis* pv. *citri,* pathogen, strain, epidemiology, disease management

Citrus canker is one of the most feared of citrus diseases, affecting all types of important citrus crops. The disease causes extensive damage to citrus and severity of this infection varies with different species and varieties and the prevailing climatic conditions. The disease is endemic in India, Japan and other South- East Asian countries, from where it has spread to all other citrus producing continents except Europe. Generally canker does not occur in arid citrus growing areas and has been eradicated from some areas. However, widespread occurrence of the disease in many areas is a continuous threat to citriculture especially in canker-free areas. Intensive research on citrus canker is being carried out throughout the world which has been reviewed by Rossetti (1977), Civerolo (1981,1984), Chand and Pal (1982), Schoulties *et al.* (1987), Stall and Civerolo (1991) and Goto (1992). However, all these reviews are either brief, restricted to one country, or by now out of date. This review aims to present an overview of citrus canker worldwide with special reference to India.

Origin and history: The geographical origin of citrus canker is a matter of controversy. Lee (1918) reported that it may have arisen in southern China, and he assumed *Fortunella hindsii* to be the wild host plant. However, Fawcett and Jenkins (1933) reported that citrus canker originated in India and Java, rather than in other regions of the Orient, because they detected canker lesions on the oldest citrus herbaria kept at the Herbaria of the Royal Botanic Gardens in Kew, England (*i.e.*, *Citrus medica* collected from India in 1827-1831 and *C.aurantifolia* from Indonesia in 1842-1844). These findings suggest 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 region 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 America (Rossetti, 1977), South Africa (Doidge, 1916) and Australia (Garnsey *et al*, 1979). The disease was reportedly eliminated in these countries as well as the Gulf States through nursery and orchard inspections, quarantines, and the on-site burning of infected trees. Subsequent epidemics have occurred in Argentina, Australia, Brazil, Oman, Saudi Arabia, Reunion Island, the USA, and Uruguay. In some locations, eradication efforts have been attempted and failed. In others, active eradication campaigns continue (Florida, Uruguay, Brazil) (Schubert and Miller, 2000).

In India, citrus occupies third position among fruits after mango and banana and canker is one of the major constraints of its cultivation. Citrus canker was first reported from Punjab (Luthra and Sattar, 1942; Bedi, 1961). Its occurrence was further recorded in Tamil Nadu (Ramakrishnan, 1954) , Andhra Pradesh (Govinda Rao,1954), Karnataka (Venkatakrishnaiah,1957; Aiyappa,1958), Rajasthan (Prasad,1959), Madhya Pradesh (Parsai,1959), Assam (Chowdhury,1951) and Uttar Pradesh (Nirvan, 1960). Several others have reported the incidence of canker on the acid lime and other varieties of citrus. Further, the disease appear as a serious problem whereever acid lime (*C. aurantifolia*) is grown on a large and commercial scale (*e.g.*, Akola region in central India, Nellore and Periyakulum regions in southern India and Khera region of western India) and has become a permanent major problem to the citrus growers of this country. Recently canker has been detected in kinnow mandarin nursery in the state of Punjab (Anonymous, 2000).

Distribution and economic importance: In spite of the heightened regulations imposed by many countries to prevent introduction,
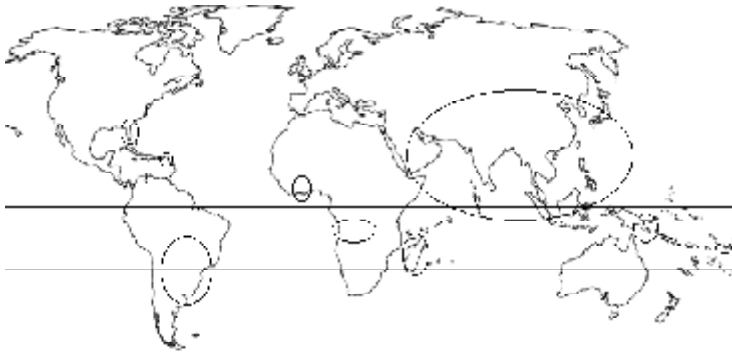
Fig. 1. Distribution map of citrus canker (*Xanthomonas axonopodis pv. citri*)

the disease continues to increase its geographic range. Citrus canker presently occurs in over thirty countries in Asia, the Pacific and Indian Ocean islands, South America, and the Southeastern USA (Fig. 1).

The economic importance of citrus canker can be analyzed from several different points of view. Loss assessment has not been determined clearly, as in the case of diseases of annual crops. 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 stages may cause only scattered canker lesions on the surface of fruits but makes fresh fruits unacceptable for market. The severity of fruit infection usually parallels that of foliage infection. Eighty to ninety percent of fruit infection is not uncommon in susceptible citrus trees that have already sustained severe foliage infection. Such heavy foliage infection often causes severe defoliation, leaving only bare twigs (Goto,1992). In Argentina, for example, 83-97% of the fruit of grapefruit trees were diseased in unsprayed plots during 1979-1980 and in the same plots, upto 88% of the leaves were infected (Stall and Seymour, 1983).

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. The disease has been studied in greater detail in the U.S. where it caused very serious damage, so much so that millions of canker affected trees were cut and burnt. In Florida, for example, during the year 1915-33, nearly 2,57,000 orchard trees and 3,000,000 nursery plants were destroyed at a cost of over $ 6 million and again during the year 1984-86, nearly 20 million citrus nursery plants were destroyed at a cost of over $ 25 million (Schoulties *et al.*, 1987). Presently over $ 12 million per year and over 600 personnel are dedicated to this programme.

In spite of this effort, the disease continues to spread in the Miami area of Florida,USA (Schubert and Miller, 2000) and hence some researchers, growers and residents are disputing the concept and feasibility of eradication.

Forms: There are three different forms of citrus canker disease caused by various pathovars and variants of the bacterium *Xanthomonas axonopodis* Starr and Garces emend. Vauterin *et al.*(1995). Differentiation of these forms is mainly based on geographical distribution and host range of the pathogen (Stall and Seymour, 1983). The Asiatic form of canker (canker A, cancrosis A or true canker), caused by *X. axonopodis* pv. *citri* (Hasse) Vauterin (***Xac***) is the most common, widespread and severe form of the disease. The disease is endemic throughout India, Pakistan, the islands of Indian Ocean, South-East Asia, China and Japan. Cancrosis B (canker B or false canker), caused by *X. axonopodis* pv. *aurantifolii* (Hasse) Gabriel Vauterin is a serious problem on lemons in Argentina, Paraguay and Uraguay. Mexican lime, sour orange, and pummelo are also susceptible. Cancrosis B causes canker-type lesions on fruit, leaves, and twigs that are similar to but smaller than those produced by the A form. In culture, cancrosis B bacteria grow more slowly than canker A bacteria on nutrient agar, and a specific medium containing sucrose, peptone, salts, and purified agar has been developed for this form. Cancrosis B isolates can be differentiated serologically from the canker A bacteria but not from Cancrosis C isolates. Cancrosis C, also caused by *X. axonopodis* pv. *aurantifolii*, has been isolated from Mexican lime in Brazil. Symptoms are the same as those of canker A. In 1984, a new xanthomonad disease of citrus was discovered in Florida nurseries. The causal bacterium is shown to have no relationship to the existing two pathovars of *Xanthomonas axonopodis* (causing canker A, B and C) and named as *Xanthomonas axonopodis* pv. *citrumelo* (Hasse) Gabriel Vauterin (earlier called group E canker or canker E). The disease is most commonly referred to as citrus bacterial spot (CBS). At present CBS is only known from Florida, where it appears to be restricted entirely to nurseries (Gottwald and Graham, 2000). The differential characteristics of the three forms of citrus canker and CBS are given in Table 1.

Other forms of citrus canker have also been reported. For example, canker D, sometimes called citrus bacteriosis, was reported in the Colima area of Mexico in 1980s (Rodriquez *et al.*,1985) but later it was found to be caused by *Alternaria limicola*. An isolate of *Xanthomonas* was discovered in Oman in 1986 that produced canker A-like lesions only on Mexican lime. Similar isolates (known as A*) have been found in Saudi Arabia, Iran, and India (Verniere *et al.*,1998). Another atypical form of canker A bacteria,

Table 1. Comparison of three different forms of citrus canker and citrus bacterial spot (CBS) of citrus

| Characteristics Canker form | Citrus Canker | | | Citrus bacterial spot (CBS) |
	A	B	C	
Pathogen	*X. axonopodis* pv. *citri*	*X. axonopodis* pv. *aurantifolii*	*X. axonopodis* pv.*aurantifolii*	*X. axonopodis* pv. *citrumelo*
Distribution	Asia, Africa,South AmericaOceania	Argentina, Paraguay, Uruguay	Brazil, Mexico	America (Florida)
Host range	Wide	Limited	Limited	Wide
Major host plant	Citrus spp.	Lemon	Mexican lime	Citrus spp. (nursery)
Symptoms	Spongy erupted at first; corky rough lesions with a raised, greasy margin later; water-soaked appearance			Flat or sunken lesion; extreme water soaking

Modified from Goto (1992)

which has high levels of resistance to penicillin related antibiotics, has been described from Reunion and surrounding islands in the Indian Ocean (Gottwald and Graham, 2000).

Symptoms: The diseased plants are characterized by the occurrence of conspicuous raised necrotic lesions that develop on leaves, twigs and fruits. Lesions can be detected by drawing the fingers over the surface of infected tissues. On leaves, first appearance is as oily looking, 2-10 mm circular spots, usually on the abaxial surface (reflecting stomatal entry following rain dispersal). Lesions are often similarly sized. Later, both epidermal surfaces may become ruptured by tissue hyperplasia induced by the pathogen. On leaves, stems, thorns and fruit, circular lesions become raised and blister-like, growing into white or yellow spongy pustules. These pustules then darken and thicken into a light tan to brown corky canker, which is rough to the touch. Often a water- soaked margin develops around the necrotic tissue and is easily viewed with transmitted light. On stems, pustules may coalesce to split the epidermis along the stem length, and occasionally girdling of young stems may occur. Older lesions on leaves and fruit tend to have more elevated margins and are at times surrounded by a yellow chlorotic halo (that may disappear as canker lesions age) and a sunken center. Sunken centers are especially noticeable on fruits, but the lesions do not penetrate far into the rind thereby not affecting internal quality. Severe infection results in defoliation, die-back, deformation of fruit and premature fruit drop (Rossetti, 1977; Civerolo, 1981; Chand and Pal, 1982; Stall and Seymour, 1983). Canker causes fruit losses ranging from premature fruit drop due to abscission to non marketable quality due to lesions. Disease of the fruit is probably the most economically important damage since fruits with canker lesion are not acceptable for fresh market and fetch very little price.

An essential diagnostic symptom of the disease is citrus tissue hyperplasia (excessive mitotic cell divisions), resulting in cankers (Gabriel *et al.,* 2000). Canker symptoms on leaves and fruit can be readily obtained by artificial inoculations. If cankers are not present on leaves, stems and fruit of mature trees, or if leaves and fruit of susceptible *Citrus* species do not develop cankers following artificial inoculation, a diagnosis of citrus canker is not indicated. Occurrence of lesions is seasonal, coinciding with periods of heavy rainfall, high temperatures and growth flushes.

Host range and varietal susceptibility: Civerolo (1984) lists a number of plants in the family rutaceae other than *Citrus* and *Poncirus* that can serve as hosts of *Xac* under experimental conditions or heavy disease pressure in nature. Among commercial citrus varieties and rootstocks, Asiatic citrus canker is most severe on grapefruit (*C. paradisi*), limes (*C. aurantifolia*, *C. limettioides*), trifoliate orange (*Poncirus trifoliata*) and their hybrids because of their high susceptibility (Table 2).

In India, citrus canker is reported to be relatively more on acid lime and less commonly on mandarin and sweet orange (Ramakrishnan, 1954). According to Aiyappa (1958) all the cultivated varieties of citrus and some wild species in Karnataka are suspectible to canker possibly due to heavy rainfall, high humidity and low temperature. Prasad (1959) from Rajasthan made similar observations. The descending order of susceptibility in citrus species is Kaghzi Lime, grape fruit, Karnakhata and sweet oranges (Nirvan, 1961). Mandarins and lemons are resistant and Kumquats are commercially immune under conditions existing in Uttar Pradesh. Jain (1959) reported that different varieties of sweet lime, grape fruit and sweet orange were infected almost to same extent in Himachal Pradesh. According to Naik (1949) acid limes, some varieties of lemon, sweet orange and grapefruit were very susceptible to canker, while Nepali oblong and round seedless lemons were highly resistant. Mundkur (1961) observed no infection in sweet orange and pummelo but Jambheri, sour orange and Kaghzi lime were very susceptible.

Host- pathogen interaction: Citrus canker research has been primarily oriented toward the ecological behaviour of the causal bacterium. Studies from physiological and biochemical standpoint are therefore very limited.

Xac produces abundant extracellular polysaccharides (EPS), both in culture media and in host tissues. The bacterial cells in canker lesions are embedded in a dense matrix of EPS and are dispersed, together with EPS, by rain splash. The EPS molecules exhibit great protective effects against the 'dilution effect' in water and desiccation in air, providing benefits for the bacterial ecology (Goto, 1985). After entering the intercellular space (through stomata or wounds) they adhere to the host cell walls through an interaction between bacterial EPS and citrus agglutinins (Takahashi and Doke, 1984). Ethylene production by citrus leaves inoculated with *Xac* and increased concentration of indole acetic acid (IAA) in the *Xac* inoculated leaves have also been reported (Goto *et al.,* 1979a).

Padmanabhan *et al.* (1973) studied the physiology of canker infected citrus leaves with special reference to halo formation, and reported that halo zone respired more than the cankered tissue. Catalase activity was very high in the halo region. Both peroxidase and ascorbic acid-oxidase activity increased in canker as well as in halo regions. They again recorded a descrease in chlorophyll a, b, carotene and xanthophyll contents in the canker, halo and pre-halo regions of the citrus leaves infected by canker-inducing bacterium. Photosynthesis was impaired in the infected regions while starch content was not affected in the halo regions (Padmanabhan

Table 2. Susceptibility of several citrus varieties and rootstocks to *Xanthomonas axonopodis* pv. citri

Highly Susceptible	Moderately Susceptible
Citrus paradisi Macf., grapefruit	*C. sinensis* (L.) Osbeck, sweet orange
C. aurantifolia (Christ.) Swingle, acid lime	*C. aurantium* L., sour orange
C. limettioides Tan., Palestine sweet lime	*C. limon* (L.) Burm., lemon
Poncirus trifoliata (L.) Raf., trifoliate orange	*C. tangelo* J. Ingram & H.E. Moore, tangelo
Moderately Resistant	**Highly Resistant**
C. reticulata Blanco, mandarin, tangerine	*C. medica* L., citron
C. maxima (Burm.) Merr., pummelo	*Citrofortunella microcarpa* (Bunge) Wijnands, calamondin
C. aurantifolia (Christ.) Swingle, Person or Tahiti lime	*Fortunella* spp., kumquat

Recently, it was reported that goat weed (*Ageratum conyzoides* L.) could serve as a host of *Xac*. This plant is common in citrus orchards in the state of Assam in India (Kalita *et al.* 1997). This represents the only report of a non-Rutaceous host of *Xac*.

et al., 1974). Total sugar content decreased in all the infected regions. Kishore and Chand (1972, 1975) carried out biochemical analysis of healthy and canker infected leaves and reported that amino acid content decreased in infected leaves. They also noticed more total phenols in resistant *C. reticulata* than in susceptible *C. aurantifolia*.

Pathogen biology

Pathogens and strains: Based on currently available information, at least three pathovars (sometimes called strains) of *Xanthomonas axonopodis* have been recognized. These pathovars are distinguished from one another by geographical distribution and by different pathogenicity to members of genus *Citrus*. The pathogen for canker A was first identified and described as *Pseudomonas citri* by Hasse (1915). Bacterial nomenclature has undergone many changes since then and the causal bacterium is now known as *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin [Syns. *X. citri* (Hasse) Dowson and *X. campestris* pv. *citri* (Hasse) Dye] (Dye *et al.,* 1980; Vauterin *et al.,* 1995). The pathogen for canker B and C and other related strains associated with the disease have already been discussed (see **Forms**).

The bacterium (*Xac*) is rod-shaped measuring 1.5-2.0 x 0.5-0.75 μm, Gram-negative, and has a single polar flagellum. Growth is obligately aerobic. Colonies on culture media are usually yellow as a result of xanthomonadin pigment production. When glucose or other sugars are added to the culture medium, colonies become very mucoid due to the production of an extracellular polysaccharide slime. The optimum temperature range for growth is 28 to 30° C (82 to 86^0 F), and the maximum temperature range for growth is 35 to 39^0 C (95 to 102^0 F). Bacterial cells are positive for hydrolysis of starch, aesculin, casein, liquefaction of gelatin, and production of tyrosinase, catalase, reducing substance from sucrose, and hydrogen sulfide. The bacterium is negative for nitrate reduction, indole production and for methyl red test (Chand and Pal, 1982; Goto, 1992).

Goto (1969) in Japan, differentiated 300 isolates of *X. citri* into 5 strains by their ability to oxidise mannitol and lactose, and by rapidity of breakdown of mannose. In Argentina, two biotypes were distinguished among 65 isolates of *Xac* based on growth on media with carbohydrates, acid production in litmus milk and colony appearance in wakimoto's medium (Falico de Alcaraz, 1980). Goto *et al.* (1980) distingushed canker A strain from the B strain by bactoriophage sensitivity test. A strains are susceptible to lysis by phage CP 1 or CP 2 while B strains are susceptible to lysis by CP 3. Civerolo and Fan (1982) successfully employed ELISA to identify the different strains of *Xac*. Alverez *et al.* (1991) produced monoclonal antibodies for A, B and C-form pathogens and noticed that canker A MAb did not react with strains associated with other forms of citrus canker (B,C).

In India, occurrence of strains (pathotypes) of the pathogen has been reported by Rangaswami and Soumini (1957) and Hamlin (1967). Khan and Hingorani (1970) grouped 15 isolates of the pathogens into 3 strains by their reaction on *Murraya exotica*. Kishore and Chand (1972) studied the reaction of eight isolates on *C. aurantifolia, C. sinensis* and *C. jambhiri* and showed the presence of more than one strain of the pathogens in Harayana. Similarly Prasad *et al.* (1978) and Buragohain and Chand (1991)

also observed strain variation in *Xac*. Recently Das (2002) reported the existence of pathogenic variability within the 'A' strain of *Xac*.

Pathogen and strain identification: Because symptoms are generally similar, identification and separation of canker pathogens and strains are based on cultural and physiological characteristics (Schaad,1988), bacteriophage sensitivity (Goto *et al.,* 1980; Civerolo, 1984), serology (Alvarez *et al.*, 1991), plasmid fingerprints (Pruvost *et al.*, 1992), DNA- DNA homology (Egel *et al.*,1991) and by various RFLP (restriction fragment length polymorphism) and PCR (polymerase chain reaction) analyses (Gabriel *et al.*, 1988; Hartung and Civerolo, 1989; Gillings *et al.,* 1995; Hartung *et al.,* 1996; Miyoshi *et al.,* 1998; Cubero and Graham, 2002). When the DNA-based assays are unavailable, strains of *Xac* can be distinguished from other pathovars by infecting a panel of susceptible and resistant citrus hosts or as a bioassay on detached-leaves or leaf-disks (Gottwald *et al.,* 1993). Such pathogenecity test is an essential component in diagnostic programmes for regulation of citrus canker diseases (Schubert *et al.*, 2001).

Pathogenecity: Identical symptoms induced by two taxonomically distinct groups of strains (canker A and B) are indicative of a common pathogenicity factor. Gene *pthA* is essential for *Xac* to elicit cankers on citrus, and *pthA* confers this ability to various *X. axonopodis* strains (for example, pathovars *alfalfae* and *citrumelo*) (Swarup *et al.,*1991; Swarup *et al.,*1992). Functionally homologous genes (*pthB* and *pthC*) have also been identified and cloned from *X. axonopodis* pv. *aurantifolii* pathotype B and pathotype C, respectively (Gabriel *et al.*, 2000). Both *pthB* and *pthC* are essential for *X. axonopodis* pv. *aurantifolii* pathotypes B and C, respectively, to cause cankers on citrus, and *pthB* and *pthC* confer this ability to various *X. axonopodis* strains. All three genes are therefore functionally interchangeable, and these genes may have been transferred horizontally on plasmids between *Xac* and *X. axonopodis* pv. *aurantifolii* strains. Genes *pthA, pthB* and *pthC* are all members of an avirulence / pathogenicity gene family widely distributed in the genus *Xanthomonas* (Swarup *et al.,*1992; De Feyter *et al.,*1993). Genes *pthA, pthB* and *pthC*, when transferred into *Xac*, *X. axonopodis* pv. *aurantifolii* or *X. axonopodis* pv. *citrumelo*, confer ability to elicit hyperplasia (cell divisions or cankers) on all citrus species in the normal host range of the recipient strain. Mutations of genes encoding either the protein injection system of the pathogen (a type III secretion system encoded by *hrp* genes) or the effector molecule, pth A/B/C, abolish pathogenicity of canker bacteria (Gabriel *et al.*, 2000).

Disease cycle and epidemiology

Survival: *Xac* survives primarily 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), but Rao and Hingorani (1963) found that the bacterium survives upto 6 months in the infected leaves. The disease is carried from season to season mainly in the cankers on twigs and branches. The pathogen can survive in diseased twigs upto 76 months (Chakravarti *et al,* 1966). Vasudeva (1958) found that the organism survived in the infected leaves for more than six months, in the sterilized soils for 52 days and in the unsterilized soils for 9 days

only. Under desiccation at 30 ^{0}C, he found the organism surviving for 11 or 12 days. Paracer (1961) observed that the bacterium was resistant to drying and was killed after 120 days in ordinary laboratory temperature.

The bacterium also survives epiphytically at lower population levels on citrus hosts without symptom development, in association with non-citrus weed and grass hosts and also in soil (Goto, 1970, 1972, Leite and Mohan, 1984). But saprophytic survival of *Xac* in soil in absence of plant tissue or debris has not been conclusively established (Goto, 1970). Graham (1989) reported that population of *Xac* have very limited survival capability in subtropical soils. Attempts to detect surviving bacteria on various inanimate surfaces such as metal, plastics, cloth and processed wood in both shade and sun indicate the inoculum dies within 24-72 hours (Graham *et al.*, 2000).

Infection: Bacterial cells ooze from existing lesions during wet weather to provide inoculum for further disease development. Infection by *Xac* occurs, like many other bacterial diseases, primarily through stomatas, and wounds produced during strong winds and by insects. Resistance of leaves, stems and fruits generally increases with tissue maturation. The period of susceptibility to wound infection may be longer than that for stomatal infection, depending on the cultivar (Goto, 1962). Lesion development and bacterial multiplication may be directly related to host resistance (Koizumi, 1979). However, the number of *Xac* cells per lesion may not always be correlated with host plant resistance (Stall *et al.,* 1980). Presence of free moisture on the host surface for 20 min. is essential for successful infection (Ramakrishnan, 1954).

Leaves, stems, and fruit become resistant to infection as they mature. Almost all infections occur on leaves and stems within the first 6 weeks after initiation of growth. Leaves are most susceptible when expanded between 50 and 80% (Filho and Hughes, 2000). The most critical period for fruit infection is during the first 90 days after petal fall. Any infection that occurs after this time results in the formation of only small and inconspicuous pustules. Because the fruit are susceptible over longer time periods than leaves, infections can result from more than one dispersal event. As a result, lesions of different ages can be found on the same fruit (Gottwald and Graham, 2000).

Dispersal: Since Xanthomonads have mucilaginous coat, they easily suspend in water and are dispersed in droplets. Spread of canker bacteria by wind and rain is mostly over short distances, *i.e.*, within trees or to neighbouring trees. Cankers develop more severely on the side of the tree exposed to wind-driven rain. Rainwater collected from foliage with lesions contains bacterial population between 105-108 cfu/ml (Goto, 1962; Stall *et al.*, 1980). If the average wind speed during rains exceeds 8 m/sec (18 mph), the disease may be very severe (Kuhara, 1978). Wind blown inoculum was detected upto 32 meters from infected trees in Argentina (Stall *et al.*, 1982). Spread over longer distances, up to 7 miles, can occur during severe tropical storms, hurricanes, and tornadoes (Gottwald *et al.*, 2001). Long-distance spread more often occurs with the movement of diseased propagating material, such as budwood, rootstock seedlings, or budded trees.

There is no record of seed transmission. Commercial shipments of diseased fruit are potentially a means of long-distance spread, but there is no authenticated record of this having happened. Nursery workers can carry bacteria from one nursery to another on hands, clothes, and equipment. Similarly, spread can also result from movement of contaminated budwood or contaminated budding equipment. Pruning, hedging, and spray equipment have been demonstrated to spread the bacteria within and among plantings. Wooden harvesting boxes that contained diseased fruit and leaves have also been implicated in long-distance spread.

Temperature between 20^0 to 30^0C with evenly distributed rains are most suitable for the disease (Ramakrishnan,1954; Reddy, 1984). As Peltier and Frederich (1926) pointed out, citrus canker is severe in regions where temperature and rainfall ascend and descend together during the year. Therefore the disease occurs in severe form in seasons and/or areas characterized by warm and humid weather conditions.

Leafminer interaction: 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. Leafminers wound leaves when they begin feeding. The feeding galleries are just below the epidermis. When the galleries become contaminated with citrus canker bacteria, numerous infections can occur, resulting in tremendous inoculum production and canker infection (Nirvan, 1961; Sohi and Sandhu, 1968; Sinha *et al.,*1972; Cook, 1988). 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 leafminer serves as a true vector of canker inoculum.

Disease management: Commercially acceptable management of canker, especially on susceptible cultivars under favourable disease development conditions, is generally difficult. 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. 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). In canker-free citrus producing areas, strict quarantine measures are practised aimed at excluding the pathogen. When the canker bacterium is introduced into such an areas (as it was in Florida, USA in 1910, 1984 and 1995) eradication campaign is conducted by uprooting and burning all suspected and infected trees. A new regulation - the "1900-ft. rule" is established recently in USA, requiring the removal and destruction of diseased citrus trees and of all healthy citrus trees within a 1900-ft radius of a diseased tree (Gottwald *et al.*, 2002).

But under endemic condition (like that which exists in India) such an eradication measure is considered not feasible. Here conditions are favourable for disease development during the major part of the year. Hence effective control of this disease depends on the continuous care and attention paid by the grower. Canker incidence under these conditions can be reduced considerably by taking integrated management approach consisting of (i) using canker-free nursery stock, (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) alongwith 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,1999, 2001).

Fawcett (1936), Naik (1949), Cheema *et al.* (1954), Ramakrishnan (1954), Govinda Rao (1954), Prasad (1959) and Paracer (1961) recommended pruning of infected twigs before the onset of monsoon and spraying of 1% Bordeaux mixture at periodical intervals for an effective control of the disease. Patel and Desai (1970) reported that pruning of affected twigs every year during Nov-Dec and 3 to 4 sprays of Bordeaux mixture (1%) in a year could reduce the disease. Two prunings alongwith 4 sprays of 5000 ppm copper oxychloride or 1% Bordeaux mixture is reported to be effective against the disease (Kishun and Chand, 1987). Other chemicals found effective against the canker were perenox (Chowdhury,1951), Ultrasulphur (Nirvan, 1961), mixture of sodium arsenate and copper sulphate (Patel and Padhya, 1964), Blitox and nickel chloride (Ram *et al.,*1972). According to Rangaswami *et al.* (1959), 500-1000 ppm streptomycin sulphate was effective when sprayed with 1% glycerine on acid lime. Six sprays of 1000 ppm streptomycin sulphate along with two prunings reduced the canker in acid lime (Balaraman and Purushotman, 1981). Other effective antibiotics were Agrimycin (Sawant *et al.,* 1985), Streptocycline (Mathur *et al.,* 1973) and Streptocycline in combination with Bordeaux mixture (Krishna and Nema, 1983). Kale *et al.* (1988), in field trials with 7 different chemicals, found that the best control of *Xac* was given by Paushamycin + Blitox followed by Bordeaux mixture. Application of neem cake solution on the foliage reduced the canker in nurseries (Dakshinamurthi and Rao, 1959; Reddy and Rao, 1960). Kale *et al.* (1994) suggested that for better control of canker, spraying of streptocycline + Copper oxychloride (0.1%) should preferably be done at 7 days or 15 days interval. Integrated application of pruning of infected twigs, Copper oxychloride (0.3%), streptocycline (100ppm) and neem cake suspension was found very effective in controlling the disease (Das and Singh, 2000). Canker incidence can also be reduced by periodic spraying of insecticides to control of leaf miner damage to newly unfolded leaves, as such damage facilitates citrus canker infection.

Control measures developed in Japan include windbreaks (Koizumi *et al.*, 1996) or pruning of diseased summer and autumn shoots, forecasting and chemical sprays. Six or seven sprays of copper are necessary to protect new growth from infection (Kuhara, 1978). In China control measure consists of spraying copper ammonium WC during summer and autumn months (Chen,1998). Gottwald and Timmer (1995) reported the efficacy of wind- breaks in reducing the spread of citrus canker in Argentina. McGuire (1988) evaluated 13 bactericidal chemicals over 3 seasons on 3 citrus species to determine their ability to control canker. In field trials conducted in Argentina, he noticed copper ammonium carbonate with 8% metallic copper was consistently superior to other products in controling *Xac*. In another field test on mature grapefruit trees, three applications per seasons of copper ammonium carbonate (CAC) or copper hydroxide + maneb were

observed to reduce lesions numbers on fruit but not on leaves (Timmer, 1988). Where copper resistance was found recommendation include addition of mancozeb to the copper sprays (Canteros, 2000). When canker occurred in the USA, the emphasis was on eradication, and other measures for control of canker were not adequately researched (Stall and Civerolo,1991). However, recently in Florida, USA, some induced systemic resistance (ISR) compounds (*e.g.* Messenger, Nutri-phite, Oxycom and FNX-100) are under evaluation for their potential to control canker A using citrus bacterial spot on swingle citrumelo as a surrogate pathosystem (Graham *et al.,* 2000).

In India, where canker disease has established since a long period it was suggested that resistant varieties and species should be grown (Mundkur, 1961). Here canker infestation is relatively more on acid lime and less common on mandarin and sweet orange. Kumquat (*Fortunella* spp.) and Hazara Narangi (*C. microcarpa*) are commonly grown in India for ornamental purpose and these are found resistant to canker. *C. latifolia* was also found to be resistant to the disease (Kishun and Chand, 1987). Although several acid lime selection/clone or hybrids have been claimed either as resistant or tolerant from different regions *e.g.* RHR-L-49 (Sai Sarbati) (Desai *et al.,*1999), Tenali (Madhavi *et al.,* 2000), ALH-77 (lime x lemon hybrid) (Prasad *et al.,* 1997), these need to be tested through multilocational trials.

Studies on biological control of citrus canker are still in a preliminary stage. Some strains of bacteria *viz.*, *Pseudomonas syringae*, *Erwinia herbicola*, *Bacillus subtilis* and *Pseudomonas fluorescence* isolated from citrus phylloplane were reported to be antagonistic *in vitro* to the canker pathogen (Ota, 1983; Goto *et al.,* 1979b; 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.

Future prospects: Citrus canker continues to be the cause of worldwide concern as a potentially hazardous threat to citriculture. There is a wide range of physiological, biochemical, serological, molecular and pathogenic variation among strains of bacteria associated with citrus canker. Moreover new strains are originating regularly as a result of mutation. A better understanding of the pathogenic specialization and proper identification of *Xac* strains are needed. This could be important also for breeding new canker resistant cultivars. The development of effective chemicals for control of citrus canker has been long claimed by citrus growers and pathologists. However, these efforts have actually been unsuccessful, as has been the case with other plant bacterial diseases in general. Most chemicals with great effectiveness *in vitro* do not necessarily show satisfactory effects. The gaps found between effectiveness *in vitro* and *in situ* may stem in part from the mode of bacterial infection. Under rainy conditions, some bacterial cells may achieve direct access to the front cavity of stomata or to wounds without being exposed to the protective chemicals left on the leaf surface. Therefore, for development of effective bactericides, emphasis must be placed on the effectiveness of chemicals reaching at least to the depth of the stomatal cavity. Recent findings have demonstrated that the plants usually carry the internal resident microbes (endophyte) in vascular systems. There is a

substantial possibility that an antagonistic microbe may be found among these endophytes which will be useful in biological control of citrus canker. Fresh approaches are also to be made to develop environmentally safe methods to combat this bacterium *viz.* search for its resistance in wild citrus and its relatives in orchards and forests of the endemic areas and application of biotechnology or genetic engineering utilizing the knowledge on its molecular mechanism of pathogenicity.

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