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Citrus Canker Approaching Century: A Review

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ABSTRACT

Citrus canker was recognized in 1912 in Florida, USA, and it became so severe that mass eradication of diseased plants was undertaken in the United States to prevent further spread. The campaign to eradicate citrus canker in the USA began in 1915 and the disease was declared eradicated from these areas by 1947. The pathogen originated in the tropical areas of Asia, such as South China, Indonesia and India, where *Citrus* species are presumed to have originated. The disease is presently prevalent in Africa, Asia, Australia, Oceania and South America. Citrus canker causes heavy losses when the infection occurs at early stages of plant growth. The causal bacterium, *Xanthomonas axonopodis* pv. *citri* (Hasse) Vauterin, has three distinct forms (A, B and C) based on geographical distribution and host range. This review focuses primarily on historical developments of canker disease, host-pathogen interactions, variability, and latest achievements in the management of the disease though quarantine, cultural means, resistance sources, biocontrol techniques and biotechnological approaches. It also takes stock of the situation where restricted chemicals are still being used in some countries for managing the disease and will be a source of information for researchers and extension workers.

Keywords: *Citrus* species, disease management, *Xanthomonas axonopodis* pv. *citri*, *X. citri*, *X. campestris* pv. *aurantifolii*

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INTRODUCTION

Citrus canker disease occurs in most citrus growing countries around the world. Although canker in citrus was recognized as a new disease in 1912 in Florida, USA, the disease may have been present in India in the 1800s. The diagnostic canker lesions in citrus are very similar to those of the fungal disease citrus scab (*Elsinoe fawcetti*) which have been noted on herbarium specimens in India as early as 1827 (Fawcett and Jenkins 1933). The disease was also described in the 1900s in South Africa (Doidge 1916) and Australia (Garnsey *et al*. 1979). Mass eradication of diseased plants was undertaken in the southern states of the USA in 1915 to prevent further spread and the disease was declared to be

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eradicated by 1947. This achievement was regarded as a rare instance of successful eradication of a plant pathogen after its establishment in an ecosystem. Subsequent epidemics have been reported in over 30 countries in Asia, Africa, Australia, Oceania and South America. Although the disease was once reported to be eradicated from the USA, Australia, New Zealand and South Africa, it once again surfaced during 1980s and was reported in Australia as well as in Mexico and Florida. These later outbreaks in Mexico in 1981 and in Florida in 1984 appear to be different from that identified in Asia (Goto 1992). A new and extensive outbreak was discovered in urban Miami, Florida in 1995. The original Miami outbreak consisted of approximately 14 square miles of infected residential properties when first discovered in September 1995, but had expanded to over 202 square miles by December 1998 (Schubert and Miller 2000). Recently, the first occurrence of the disease has been reported from Somalia (Balestra 2008) and Koulikoro Province of Mali (West Africa) where canker symptoms have been observed on limes, sweet oranges, tangerines and sour oranges and disease incidences was 50, 15, 24 and 25%, respectively (Traore *et al*. 2008).

In India, citrus canker was first reported in Punjab in 1940 (Luthra and Sattar 1940) and now the disease is known to occur in almost all citrus growing areas of the country (Gupta and Sharma 2000) such as Assam (Chowdhury 1951), Andhra Pradesh (Govinda Rao 1954), Karnataka (Venkatakrishnaiah 1957; Aiyappa 1958), Madhya Pradesh (Parsai 1959), Rajasthan (Prasad 1959), Uttar Pradesh (Nirvan 1960) and Tamil Nadu (Ramakrishnan 1954). The pathogen probably originated in the tropical areas of Asia, such as South China, Indonesia and India where citrus species are presumed to have originated.

At least 3 distinct forms or types of citrus canker are recognized. Among these, the Asiatic form (Canker A), is the most destructive and affects most 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. In countries free of the disease, quarantine or regulatory programmes 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, are in effect. 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 review focuses primarily on the historical developments of canker disease, host-pathogen interactions, variability, and latest achievements in the management of the disease though quarantine, cultural means, resistance sources, biocontrol techniques and biotechnological approaches (Gottwald *et al*. 2002; Yang *et al*. 2002; Das 2003). The review also examines the use restricted chemicals in some countries for the control of citrus canker. This comprehensive review attempts to integrate different aspects of disease development which will act as source for generation of future research by professionals involved in both research and extension.

ECONOMIC IMPORTANCE

Citrus canker is a highly contagious disease caused by the bacterium, *Xanthomonas axonopodis* pv. *citri*. An infestation can destroy entire orchard crops, but the disease poses no health risk to humans or animals. It can be a serious disease where rainfall and warm temperatures are prevalent during periods of shoot emergence and early fruit development. Citrus canker is mostly a leaf spotting and fruit rind blemishing disease, but when conditions are highly favorable for infection, infections cause defoliation reducing fruit quality and quantity, shoot dieback, and fruit drop. Citrus canker seriously limits citrus production in Asia and South America. The disease causes heavy losses when the infection occurs at early stages of plant growth (Gupta and Sharma 2008). The fruits crack or become malformed as they grow and the heavily infected fruits fail to develop and fall from the tree prematurely. Severe foliage infection often causes defoliation, leaving only the bare twigs leading to almost complete loss (Goto 1992). In heavily infested areas, canker also causes such losses to grapefruit, sweet orange and lime. There is no cure and resistance cannot be genetically introgressed by breeding. This is especially the case where tropical storms are prevalent. Worldwide millions of dollars are spent annually on prevention, quarantine, eradication programmes and disease control. Undoubtedly, the most serious consequence of citrus canker infestations is the impact on commerce resulting from restrictions to interstate and international transport and sale of fruit originating from infested areas (Das 2003).

HOST RANGE

All cultivated species of Rutaceae are susceptible to citrus canker, such as *Citrus* spp., *Fortunella* spp., and *Poncirus* spp., cultivars, hybrids of citrus and citrus relatives including orange, grapefruit, pummelo, mandarin, lemon, lime, tangerine, tangelo, sour orange, rough lemon, calamondin, trifoliate orange and kumquat. In general, in field plantations, grapefruit, Mexican limes, and trifoliate orange are highly susceptible to canker; sour orange, lemon, and sweet orange are moderately susceptible; and mandarins are moderately resistant. Within orange cultivars, early maturing cultivars are more susceptible than mid season cultivars, which are in turn more susceptible than late season cultivars. However, when plant tissues are disrupted by wounds or by the feeding galleries of the Asian leafminer (*Phyllocnistis citrella* Stainton), internal leaf tissues (mesophyll) are exposed, then all cultivars and most citrus relatives that express some level of field resistance can become infected. In India, citrus canker icidence is more on acid lime as compared to mandarin and sweet orange (Ramakrishnan 1954). In artificial inoculations, at least race-specific avirulence may account for the host range differences between pathotypes B and C of *X. campestris* pv. *aurantifolii*. Experimental inoculations of *X. axonopodis* pv. *citri* in different tissues of Tahiti lime (*Citrus latifolia*) and pineapple sweet orange (*Citrus sinensis*) with respect to Asiatic citrus canker (ACC) disease expression, area under the disease progress curve (AUDPC), inoculation date (Id), fruit and leaf age ratings (FAR and LAR), and number of days during the first 2 weeks post-inoculation for which the temperature was less than 14°C or more than 28°C has shown impacts on ACC epidemiology according to the tissues involved (Vernière *et al*. 2003).

SYMPTOMS

The symptoms of the disease are observed on all the aerial parts including leaves, twigs and fruits. Occurrence of lesions is seasonal, coinciding with periods of heavy rainfall, high temperatures and growth flushes. These factors generally coincide with early summer in citrus growing regions where rainfall increases as temperatures increase. Citrus canker is unlikely to be found in regions where rainfall decreases as temperatures increase. Although phylogenetically different strains of *Xanthomonas* cause citrus canker, the symptoms and signs elicited on susceptible hosts are the same. The disease symptoms as a whole are described as follows.

Leaf lesions

Citrus canker lesions start appearing after 15-20 days after bud burst (Zhong and Ling 2002) as pinpoint oily looking spots and attain a maximum size of 2 to 10 mm circular spots usually on the abaxial surface. The eventual size of

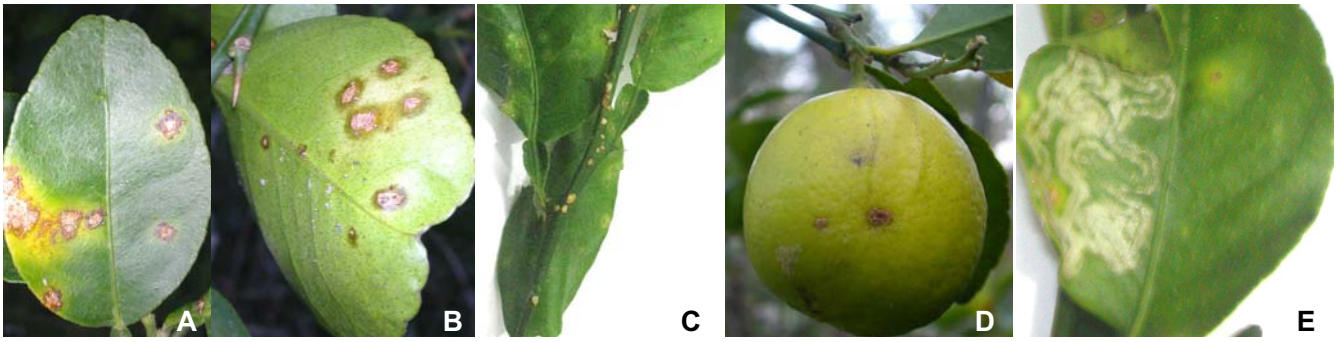

Fig. 1 Different types of lesions produced on different plant parts of citrus cv. "Kagzi". (**A**) Yellow halo and raised lesions on upper leaf surface; (**B**) Coarse raised lesions on lower leaf surface; (**C**) Lesions on twig; **D**) Lesions on fruit; (**E**) Leaf minor interactions.

the lesions depends mainly on the age of the host tissue at the time of infection and on the citrus cultivar. Lesions become visible about 7 to 10 days after infection on the underside of leaves and soon thereafter on the upper surface. The young lesions have a coarse raised surface, but particularly on the lower leaf surface (**Fig. 1A, 1B**). The pustules eventually become corky and crateriform with a raised margin and sunken center. Later, both epidermal surfaces may become ruptured by tissue hyperplasia induced by the pathogen, resulting in the formation of the diagnostic symptom. Old lesions sometimes fall out, leaving behind a scattering of round holes. Initially, the lesions are surrounded by a yellowish halo (**Fig. 1A**). A more reliable diagnostic symptom of citrus canker is the water-soaked margin that develops around the necrotic tissue, which is easily detected with transmitted light. Signs of the pathogen are generally evident in older lesions as masses of rod shaped bacteria streaming from the edges of thinly cut lesion sections under the microscope.

Twig lesions

The cankers are irregular, rough becoming white or yellow pustules and more prominent on twigs and branches (**Fig. 1C**). On stems, lesions can remain viable for several seasons. Thus, stem lesions can support long-term survival of the bacteria. These pustules may coalesce to split the epidermis along the stem length, and occasionally girdling of young stems may occur (Das 2003).

Fruit lesions

On the fruits, the lesions are almost similar to those on leaves and have a crater like depression in the centre and extend to 1 mm in depth. The lesions can vary in size because the rind is susceptible for a longer time than leaves and more than one infection cycle can occur. With time such lesions become rough and raised and develop a brown to dark brown colour (**Fig. 1D**). Infection of fruit may cause premature fruit drop, but if the fruit remains on the tree until maturity, such fruit have reduced fresh fruit marketability. Usually the internal quality of fruits is not affected, but occasionally individual lesions penetrate the rind deeply enough to expose the interior of the fruit to secondary infection by decay microorganisms. Further, the presence of a large number of lesions on the fruit surface may result in small and misshapen fruits especially when the infection is early. Defoliation and premature abscission of affected fruit occurs on heavily infected trees (Stall and Seymour 1983).

Leaf miner interaction

The Asian leafminer (*Phyllocnistis citrella*) 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 (**Fig. 1E**). Leafminers feed on the epidermis just below the leaf cuticle. Numerous cracks occur in the cuticle covering leafminer galleries providing means for bacteria to penetrate directly into the palisade parenchyma and spongy mesophyll, which are highly susceptible to infection. Citrus foliar wounds normally callus within 1-2 days; however, the extensive wounds composed of the entire leafminer feeding galleries do not callus for 10-12 days, greatly extending the period of susceptibility of galleries to infection. Leafminer infestations can be very severe producing hundreds of potential infection courts on individual trees. When bacterial dispersal occurs in the presence of the leafminer, not only is inoculum production greatly exacerbated, but so is the potential for infection over the entire dispersal range. Higher incidence of diseased plants, area under the disease progress curve, disease severity and shorter incubation periods were observed in plants inoculated after leaf minor infestation. These factors explain the association found between the higher citrus canker intensity and the damage caused by the insect and show, albeit partially, the consequences of these changes in the spread of the pathogen under natural conditions of infection (Jesus Jr. *et al*. 2006).

Interest in the interaction between the citrus leafminer and citrus bacterial canker has increased as a greater incidence and severity of canker diseased plants is observed in groves infested with the citrus leafminer. To determine whether adults of the citrus leafminer could act as vectors of citrus canker, Belasque Jr. *et al*. (2005a) tested two potential mechanisms for direct spread by leafminer adults using experimental microcosms. First, adult leafminers were raised on canker infected foliage and were allowed to mate and lay eggs on healthy plants. These plants then were observed for development of citrus canker symptoms. In a second set of experiments, adults raised on healthy plants were given free access to canker diseased plants during the period in which they mated and laid eggs on healthy plants. In all, 3,119 mines were produced by developing larvae on a total of 2,384 leaves examined for citrus canker symptoms. No symptoms of citrus bacterial canker disease were observed on any of the healthy test plants in 37 independent experimental trials conducted to test these two potential mechanisms of spread of citrus canker and the pathogen was not recovered from insects exposed to symptomatic 'Rangpur' lime plants. The upper limit on the rate of transmission was estimated to be less than 0.2% per oviposition event based on the binomial probability distribution. However, when adult *P. citrella* insects were artificially contaminated with high levels of *X. axonopodis* pv. *citri*, transmission to 'Rangpur' lime plants with the induction of citrus canker was observed. This suggests that the ability of *P. citrella* to transmit *X. axonopodis* pv. *citri* is limited by the rate at which it can acquire inoculum from infected plants. The results support the conclusion that adult citrus leafminers are not efficient vectors for citrus canker bacteria and the disease is unlikely to be spread this way (Belasque Jr. *et al*. 2005).

However, a significant relationship between leafminer damage and the incidence of citrus canker has been observed. It was also found that the intensity of canker spots

were more in leafminer affected leaves (Saravanan and Savithri 2003). Plants inoculated with 2nd and 3rd instar larvae or pupae showed high percentages (94.3, 98.3 and 100%, respectively) of bacterium infected leaves. The damage caused by this insect was responsible for the increase in citrus canker infestation. The leaf infection rate by *X. axonopodis* pv. *citri* on pre-injured leaves was similar to that observed on mechanically damaged leaves inoculated with the bacterium, with 94.1 to 97.0% of the leaves presenting bacterial pustules (Chagas *et al*. 2001).

BIOLOGY OF PATHOGEN

Causal organism

The genus *Xanthomonas* is a diverse and economically important group of bacterial phytopathogens, belonging to the gamma subdivision of the Proteobacteria. *X. axonopodis* pv. *citri* (Xac) (Hasse) Vauterin [Syns. *X. citri* (Hasse) Dowson and *X. campestris* pv. *citri* (Hasse) Dye] (Dye *et al*. 1980; Vauterin *et al*. 1995) causes citrus canker, which affects most commercial citrus cultivars, resulting in significant losses worldwide (da Silva *et al*. 2002). The bacterium is rod shaped measuring 1.5-2.0 × 0.5-0.75 µm, Gram-negative, and has a polar flagellum. Colonies on laboratory media are yellow due to 'xanthomonadin' pigment production. When glucose or other sugars are added to the culture medium, colonies become very mucoid due to the production of exopolysaccharide slime. The optimum temperature range for growth is 28 to 30°C and maximum temperature ranges for growth is 28 to 39°C (Goto 1992).

Isolation

The pathogen may be isolated and cultured from all affected plant tissues by commonly used methods. Lesions are excised with a scalpel or razor, washed with tap water, surface sterilized for 3 minutes in a 10% dilution of commercial hypochlorite bleach, rinsed and sectioned. The water-soaked tissue at the lesion margin is dragged across a sterile agar medium containing 50 ppm kasugamycin. *X. citri* strains grow well on various nutrient agar media containing: 0.5% tryptone, 0.3% yeast extract, 0.09% $CaCl_2$, 0.05% K_2HPO_4 and 1.5% agar in tap water, pH 7.2 (Gabriel *et al.* 1989). Yellow mucoid colonies generally appear within 48 hours. *X. campestris* pv. *aurantifolii* strains are reportedly difficult to isolate and culture directly from citrus tissue; these strains may be cultured initially on 1% sucrose, 0.5% peptone, 0.05% K_2HPO_4, 0.03% $MgSO_4$ and 2% Difco purified agar (Canteros 1985). After initial culturing, however, these strains appear to adapt to other media and may be routinely cultured on nutrient media (Das 2003).

Identification and detection

Identification and detection of the canker pathogen and strains are done with the help of cultural and physiological characteristics, bacteriophage sensitivity, serology, plasmid fingerprints, DNA-DNA homology, RFLP and PCR. Colonies on agar plates are circular, convex, semi-translucent and yellow, and the margins are entire and standard determinative tests are used to identify strains of the genus (Schaad 1988; Rudolph 1990). Crude methanol extracts (10 minutes at 65°C) of cells exhibit a major absorption peak between 443 and 446 nm (Gabriel 1989), which is diagnostic of the xanthomonadin pigment and not found in other yellow bacteria. Bacterial cells are positive for hydrolysis of starch, aesculin, casein, liquification 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 the methyl red test (Chand and Pal 1982; Goto 1992). Goto (1969) categorized 300 isolates of *X. citri* into 5 strains on the basis of their ability to oxidise mannitol and lactose and by rapid breakdown of mannose. In Argentina, two biotypes were distinguished among 65 isolates of *X. citri* 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) distinguished canker strains by a bacteriophage sensitivity test. 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 *Xa*c. 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 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 exotic*a. 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 Haryana. Similarly Prasad *et al*. (1978) and Buragohain and Chand (1991) also observed strain variation in the pathogen.

All strains of *X. citri* form a clonal group where as strains of *X. campestris* pv. *aurantifolii* form a different clonal group; the groups may be identified and distinguished from all other xanthomonads by characteristic restriction fragment length polymorphism (RFLP) profiles. A detailed protocol on this identification technique and its application to *Xanthomonas* has been published Gabriel and Feyter 1992. The use of RFLP data alone to formulate taxonomy and reinstate *X. citri* to species has been criticized (Vauterin *et al*. 1990), but the reinstatement was not invalidated. Most microbial taxonomists agree that phylogeny should determine taxonomy and that "the phylogenetic definition of a species generally would include strains with approximately 70% or greater DNA-DNA relatedness" (Wayne *et al*. 1987). Since *X. citri* strains are only 30% similar to *X. campestris* 33913 (the type strain), the DNA-DNA hybridization data are consistent with the RFLP data, and the reinstatement to species is consistent with the phylogenetic standard. The taxonomic status of *X. campestris* pv. *aurantifolii* strains is unresolved. These strains are only 37-40% related to *X. campestris* 33913, but are also only 62-63% related to *X. citri* strains, form a distinct RFLP group (Gabriel 1989), and differ serologically from *X. citri* strains. The causal agents currently are classified as pathovars *citri* ("A"), *aurantifolii* ("B/C/D") and *citrumelo* ("E") of a single species, *X. campestris* pv. *citri* (or *X. axonopodis* pv. *citri*). Schaad *et al*. (2005) reported that under stringent DNA reassociation conditions (Tm-15 degrees C), there are three distinct genotypes of citrus pathogens viz. taxon I which included all "A" strains; taxon II contained all "B", "C", and "D" strains; and taxon III contained all "E" strains. Hence, they proposed taxa I, II, and III citrus strains be named, respectively, *Xanthomonas smithii* subsp. *citri* (ex Hasse, 1915) sp. nov. nom. rev. comb. nov., *Xanthomonas fuscans* subsp. *aurantifolii* (ex Gabriel *et al*. 1989) sp. nov. nom. rev. comb. nov., and *Xanthomonas alfalfae* subsp. *citrumelo* (ex Riker and Jones) Gabriel *et al*. 1989 nov. rev. comb. nov.

Identical symptoms induced by two taxonomically distinct groups of strains are indicative of a common pathogenicity factor. Gene *pthA* is essential for *X. citri* to elicit cankers on citrus, and *pthA* confers this ability to various *X. campestris* strains (Swarup *et al*. 1991, 1992). Functionally homologous genes (*pthB* and *pthC*) have also been identified and cloned from *X. campestris* pv. *aurantifolii* pathotype B and pathotype C, respectively. Both *pthB* and *pthC* are essential for *X. campestris* pv. *aurantifolii* pathotypes B and C, respectively, to cause cankers on citrus, and *pthB* and *pthC* confer this ability to various *X. campestris* strains. All three genes are therefore functionally interchangeable and these genes may have been transferred horizontally on plasmids between *X. citri* and *X. campestris* pv. *aurantifolii* strains. Apparently homologous genes are found in all canker causing strains and have not been found in non-canker

inducing strains isolated from citrus, such as *X. campestris* pv. *citrumelo*. Therefore a single common gene appears to be diagnostic for a *Xanthomonas* strain's ability to induce cankers on citrus.

Genes *pthA*, *pthB* and *pthC* are all members of an avirulence/pathogenicity gene family widely distributed in the genus *Xanthomonas* (Swarup 1992). Avirulence genes determine race specificity and can determine host range (Gabriel and Rolfe 1990). Genes *pthA*, *pthB* and *pthC*, when transferred into *X. citri*, *X. campestris* pv. *aurantifolii* or *X. campestris* pv. *citrumelo*, confer ability to elicit hyperplasia on all citrus species in the normal host range of the recipient strain. Pathotype B of *X. campestris* pv. *aurantifolii* causes "false" citrus canker or cancrosis B, while pathotype C causes 'Mexican lime' cancrosis or cancrosis C.

Coletta-Filho *et al*. (2005) has designed two primers, Xac01 and Xac02, which provide specific and sensitive detection of *X. campestris* pv. *aurantifolii* in all citrus tissues where the pathogen is found. This PCR-based diagnostic test is suitable for monitoring asymptomatic plants in areas where the bacteria is endemic, in plant quarantine and regulatory situations, and also for obtaining an accurate diagnosis in a very short time.

Recently in Wellington and Lake Worth areas of Palm Beach County, FL, citrus canker appeared on Key/Mexican lime (*Citrus aurantiifolia*) and alemow (*C. macrophylla*) trees over a period of about 6 to 7 years before detection, but nearby canker susceptible citrus, such as grapefruit (*C.* x *paradisi*) and sweet orange (*C. sinensis*), were unaffected (Sun *et al*. 2004). Colonies of the causal bacterium, isolated from leaf, stem, and fruit lesions, appeared similar to the Asiatic group of strains of *X. axonopodis* pv. *citri* (Xac-A) 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*), Orlando tangelo (*C. reticulata* × *C.* x *paradisi*), and trifoliate orange (*Poncirus trifoliata*). The bacterium did not react with the Xac-A specific monoclonal antibody A1 using enzyme-linked immunosorbent assay (ELISA) and could not be detected by polymerase chain reaction (PCR) based assays using primers selected for Xac-A. DNA reassociation analysis confirmed that the pathogen, designated as Xac-AW, was more closely related to Xac-A and Xac-A* strains than *X. axonopodis* pv. *aurantifolii* or the citrus bacterial spot pathogen (*X. axonopodis* pv. *citrumelo*). The strain can be easily differentiated from Xac-A and Xac-A* using ELISA, PCR based tests, fatty acid analysis, pulsed-field gel electrophoresis of genomic DNA, and host specificity (Sun *et al*. 2004). The phenylacetaldehyde *O*-methyloxime may potentially be used to identify citrus bacterial canker disease (CBCD) infestations. However, more intensive studies will be required to fully evaluate the potential of phenylacetaldehyde *O*-methyloxime as a diagnostic compound for citrus bacterial canker disease CBCD. Using solid phase micro extraction (SPME) and gas chromatography-mass spectrometry (GC-MS) to measure phenylacetaldehyde *O*-methyloxime may provide an easy and feasible tool to complement current methods used to detect *X. axonopodis* pv. *citri* in environmental samples (Zhang and Hartung 2005). An integrated approach for reliable detection of *X. axonopodis* pv. *citrumelo* 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 bacterium on fresh fruits (Golmohammadi *et al.* 2007). New *Xanthomonas* isolates causing citrus bacterial canker in Korea were differentiated primarily on the basis of host range by comparison with reference strains. The new isolates were pathogenic to *Citrus sinensis*, *C. paradisi*, *C. limon* and *C. unshiu* and formed crater-like canker on the plants; this indicated that they were *X. axonopodis* pv. *citri* A types. Further molecular characterization using rep-PCR fingerprinting and 16S rDNA sequence analysis and cluster analysis by combining the band patterns of ERIC-, BOX- and REP-PCR clearly separated one group including only *X. axonopodis* pv. *citrumelo* and the other group including *X. axonopodis* pv. *citri* and X. *axonopodis* pv. *aurantifolii* strains. There was a clear separation between *X. axonopodis* pv. *citri* Asiatic types and *X. axonopodis* pv. *aurantifolii* B, C types in the second group. Partial sequence analysis of 16S rDNA revealed that all strains of *X. axonopodis* pv. *aurantifolii* B and C type, and *X. axonopodis* pv. *citrumelo* formed a distinct cluster with a similarity of 99%. The results indicate that the isolates causing citrus canker in Korea belong to the A type of *X. axonopodis* pv. *citri* (Lee *et al*. 2008).

Pathogenicity and host interactions

Recovery of *X. citri* on agar media is generally not a problem and these strains do not lose virulence readily upon subculturing. Bacteria may be grown in liquid culture or scraped off a freshly streaked agar plate and suspended in tap water for inoculation into citrus. Recovery of *X. campestris* pv. *aurantifolii* strains on agar media can be a serious problem. Once cultured, bacteria may be harvested for inoculation as above. If axenic culturing of bacteria proves difficult, the lesions should be excised and ground in a mortar and pestle in several milliliters of tap water. After debris has settled, the crude bacterial suspension may be directly inoculated.

Pathogenicity tests should be conducted on younger leaves using control strain(s) if possible. For either direct inoculations from citrus, or inoculations from culture, the bacterial suspension should be drawn into a tuberculin syringe, the blunt end of the syringe appressed gently, but firmly against the abaxial citrus leaf surface and the slurry forced into the stomata until about two cm^2 of the leaf is water congested. The congestion is transient and disappears within a few minutes. A control strain grown under the same conditions as the test strain(s) should be inoculated into the same leaf, on the other side of the mid-vein. Six different strains may be conveniently inoculated onto the same leaf, three on each side of the mid-vein.

The key diagnostic symptom is tissue hyperplasia. Symptoms are generally first observed four days after inoculation as a raised margin surrounding a slightly chlorotic region. Over time, the raised margin becomes pronounced, roughened and corky, while the central region of the lesion becomes necrotic and collapsed. After several weeks, the necrotic lesions may split and the leaves abscise. If pathotype C of *X. campestris* pv. *aurantifolii* is inoculated on an incompatible host, the hypersensitive response appears within 48 hours and leaves typically abscise several days later. On Mexican lime, cankers should be observed.

X. campestris pv. *aurantifolii* strains are reportedly difficult to isolate and culture directly from citrus tissue; these strains may be cultured initially on 1% sucrose, 0.5% peptone, 0.05% K_2HPO_4, 0.03% $MgSO_4$ and Difco purified agar. After initial culturing, however, these strains appear to adapt to other media and may be routinely cultured on other nutrient media. Diagrammatic scales are important tools for disease severity assessment. Four diagrammatic scales for isolated small (SL), medium (ML), and large (LL) lesions and for symptoms associated with the leaf miner injuries (LM) were developed to standardize the severity assessments of citrus canker caused by *X. axonopodis* pv. *citri* on leaves of citrus (Belasque Jr. *et al*. 2005b).

The participation of the *X. axonopodis* pv. *citri* hypersensitive response and pathogenicity (hrp) cluster in interactions with host and non host plants has been characterized in pathogenicity and avirulence models. The hypersensitive response is activated in leaves of cotton, bean, tobacco, tomato, pepper and *Nicotiana benthamiana*, and those genes present in operons hrpB and hrpD and the hrpF gene are required for pathogenicity in hosts and induction of the hyper-

sensitive response in non host plants (Dunger *et al*. 2005).

Telomerase (TERT), a specialized reverse transcriptase, mediates maintenance of telomere length and is closely associated with cellular proliferation capacity. Because disordered cell division and cell enlargement are crucial events for symptom development with citrus canker, the involvement of telomerase activity was recorded specifically in citrus leaves infected with *X. axonopodis* pv. *citri*, but not in mock-inoculated leaves, indicating a possible role for telomerase in citrus canker development (Ishihara *et al*. 2004). *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*. 1979).

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. Photosynthesis was impaired in the infected regions, while starch content was not affected in the halo regions (Padmanabhan *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.

Das (2002) has reported the pathogenic variability amongst twelve isolates of *X. axonopodis* pv. *citri* collected from acid lime (*Citrus aurantiifolia*), rough lemon (*C. macrophylla*) and trifoliate orange (*Swingle citrumelo*) from Maharashtra, Karnataka, Andhra Pradesh and Tamil Nadu, India. The bacterial isolates *Xac*2 and *Xac*6 from Maharashtra were the most pathogenic to acid lime causing up to 75% canker severity. *Xac*11 from Tamil Nadu was the least pathogenic, causing only 1-10% canker severity on acid lime, rough lemon and trifoliate orange.

Storage of bacterium

Strains may be stored by lyophilization, by freezing, with silica gel or in sterile tap water. For freezer storage, media containing 15% glycerol is suitable and strains may be held at -80°C or in liquid nitrogen. In silica gel storage, bacteria are suspended in 0.5 ml of 10% aqueous dry milk powder and mixed with 3 g sterilized anhydrous silica gel in chilled storage tubes (Sleesman and Leben 1978).

A very convenient method is storage in sterile tap water. Tap water containing high levels of calcium is most appropriate; deionized or distilled water is not suitable. Several loopful of bacteria may be scraped off a freshly streaked agar plate, suspended in 2 ml of sterile tap water, and stored at room temperature for many years in screw capped vials with a teflon seal. Strains die within six weeks on all agar media tested, whether refrigerated or not.

Pathogen diversity and distribution

Serology, host range, cultural and physiological characteristics, bacteriophage typing, fatty acid profiles, PCR and DNA analysis are useful for identification and classification of bacterial isolates into pathovars. Citrus canker disease has been historically described as having different "forms". However, these three "forms" are not distinctive in terms of disease phenotype, and have not been distinguished based upon host symptoms. Differentiation of these forms is mainly based on geographical distribution and host range of the pathogen (Stall and Seymour 1983), however, other unrecognized strains may also exist. At least 3 distinct forms or types of citrus canker viz. Asiatic or cancrosis A, false citrus canker or cancrosis B and Mexican lime cancrosis or cancrosis C have been recognized (Vauterin *et al.* 1995).

Amongst these, Asiatic citrus canker (Canker A) caused by *X. axonopodis* pv. *citri* (Hasse) Vauterin (*Xac*) is the most destructive and affects most of the citrus cultivars, most common, widespread and severe form of the disease. The "A" strain affects members of the plant family Rutaceae, including most citrus species and hybrids, especially grapefruit, lime, sweet lime, and trifoliate orange (Goto 1992). The current and all previous U.S. infestations have been associated with the "A" strain. Cancrosis B (canker B or false canker), caused by *X. axonopodis* pv. *aurantifolii* (Hasse) Gabriel Vauterin is a serious problem on lemons, Mexican lime, sour orange, and pummelo. Cancrosis B causes canker-type lesions on fruit, leaves, and twigs that are similar to, but smaller than those produced by the A form. It grows more slowly than canker on culture media. Cancrosis B isolates can be differentiated serologically from the canker A bacteria, but not from Cancrosis C isolates. This strain affects lemons in Argentina, Uruguay, and Paraguay. However, Mexican or key lime, sour orange, Rangpur lime, sweet lime, citron, and occasionally sweet orange and mandarin orange can also be affected. Cancrosis C, also caused by *X. axonopodis* pv. *aurantifolii*, has been isolated from Mexican lime. Symptoms are the same as those of canker A. The only other known host for this bacterium is sour orange.

In addition to these three forms of citrus canker, D and E forms have also been reported which have no relationship to the existing strains and named as *X. axonopodis* pv. *citrumelo* (Hasse) Gabriel Vauterin. The disease caused by E form is most commonly referred to as citrus bacterial spot (CBS). At present CBS is only known in Florida, where it appears to be restricted entirely to nurseries (Gottwald and Graham 2000). The causal agents currently are classified as pathovars *citri* ("A"), *aurantifolii* ("B/C/D") and *citrumelo* ("E") of a single species, *X. campestris* pv. *citri* (or *X. axonopodis* pv. *citri*) (Schaad *et al*. 2005).

DISEASE CYCLE AND EPIDEMIOLOGY

Seasonal carry over

Since citrus is a perennial plant, there is no problem for the survival of the bacterium, which easily over winters on naturally occurring cankered lesions on the leaves, stems, twigs and fruits. The bacteria remain alive in the margins of the lesions in leaves and fruit until they abscise and fall to the ground. The bacterium survives up to 6 months in the infected leaves (Rao and Higorani 1963). Bacteria have also been reported to survive in lesions on woody branches up to a few years of age. The pathogen can survive in diseased twigs up to 76 months (Chakravarti *et al*. 1966). Bacteria may also survive in crevices in the bark tissues of citrus trees. Bacteria that ooze onto plant surfaces do not survive and begin to die upon exposure to rapid drying due to direct sunlight. Survival of exposed bacteria is limited to a few days in soil and to a few months in plant refuse that is incorporated into soil. Bacterial populations appear to decline rapidly in soil. On the other hand, the bacteria can survive for years in infected plant tissues that have been kept dry and free of soil. It has been suggested that the bacterium may survive at low population levels on citrus hosts without developing symptoms, and it may also survive for short periods of time on some weeds and grasses however, these survival mechanisms require confirmation. Xanthan produced by the bacterium does not play an essential role in citrus canker at the initial stages of infection or in the incompatible interactions between *X. axonopodis* pv. *citri* and non-host plants, but facilitates the maintenance of bacteria on the host plant, possibly improving the efficiency of colo-

nization of distant tissue (Dunger 2007).

Infection and disease development

The relationship between citrus canker severity and leaf wetness duration has been explained by a monomolecular model. The greatest severity occurs at 24 h of leaf wetness, with 4 h of wetness being the minimum duration sufficient to cause 100% incidence at optimal temperatures of 25-35°C; however, the estimated minimum and maximum temperatures for the occurrence of disease are 12 and 40°C, respectively (Pria *et al*. 2006). The occurrence of citrus canker has a close relationship with the daily mean temperature: when a daily temperature of 12°C occurs for 10-15 days, the spring shoots and fruitlets will be attacked (Zhong and Link 2002). Canker develops more severely on the side of the tree exposed to wind-driven rain which is the main dispersal agent and wind ≥ 8 m/s (18 mph) aids in the penetration of bacteria through the stomatal pores or wounds made by thorns, insects (through leafminer, *Phyllocnistis citrella*, Nirvan 1961) and blowing sand. Populations of *X. axonopodis* pv. *citri* in leaf and twig lesions are the most important inoculum source for secondary infections. Almost all infections occur on leaves and stems within the first six weeks after initiation of growth. The most critical period for fruit rind 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.

Dissemination

The bacterial cells start multiplying inside the host tissue during the onset of spring, ooze out in large numbers and spread locally primarily by wind-driven rain, air currents, overhead irrigation, flooding, insects, birds, human movement within groves and contaminated equipment. Spread over longer distances, up to several miles, results from severe meteorological events, such as tropical storms, hurricanes, and tornadoes. However, long-distance spread more often occurs with the movement of infected plants, seedlings, propagative material, such as budwood, rootstock seedlings, or budded seedlings and fruit and are the primary means of spreading the canker pathogen. There is no record of seed transmission. Commercial shipments of diseased fruit are potentially a means of long-distance spread. Contaminated clothing, tools, packing boxes, and other items associated with harvesting and post harvest handlings of fruits are also potential sources of infection. Nursery workers can carry bacteria from one nursery to another unless hands, clothes, and equipment are disinfected. Such spread can also result from contaminated bud wood or contaminated budding equipments. Pruning, hedging, and spray equipment have been demonstrated to spread the disease within and among plantings. Leaves, stems, and fruits become resistant to infection as they mature unless they are wounded. The first flush in spring is infected by the pathogenic bacterium splashed by rains from the canker lesions on the over-wintered shoots. The disease on the spring shoots may be limited to a rather short period of time unless the leaves are injured by storms (Goto 1992), but on angular shoots that develop from summer to autumn, the disease may continue for several months because of the availability of young, susceptible shoots for a long time. Because the fruit are susceptible over longer periods compared to leaves, infections can result from more than one dispersal event resulting in lesions of different age on the same fruit. It is helpful in estimating when infection has occurred and can be correlated to meteorological events, such as storms, that occurred at that time. The bacteria enter the plant tissue through stomata on leaves or small wounds created due to thorn bruising and insects. Multiplication of bacteria occurs mostly while the lesions are still expanding and the number of bacteria produced per lesion is related to general host susceptibility. Although heavily infected leaves defoliate in winter, lesions on the stem or on slightly infected attached leaves become the major inoculum source in the following spring. Late infection in autumn often remains latent and the pathogen becomes active in the next season. The disease seems to be much more severe in areas experiencing high rainfall with high mean temperature. The highest incidence of citrus canker (73.3%) and scab (66.6%) was recorded during the second week of September. Both diseases showed a positive correlation with temperature, relative humidity and rain. The period from July to September was identified as the most conducive for the development of citrus canker and scab (Bal and Dhiman 2005). Citrus canker is readily dissersed in wind-driven rain and is dispersed in large quantities immediately after the stimulus occurs, upon which wind-driven splash can disperse inoculum over a prolonged period and over a substantial distance (Bock *et al*. 2005). Out of different environmental variables, minimum temperature and wind speed significantly influenced the citrus canker disease development and a multiple regression model consisting of these two variables explained 92% of the variability in disease development (Khan *et al*. 2002).

MANAGEMENT

Quarantine measures

In canker-free citrus producing areas, strict quarantine measures are practiced to exclude the pathogen. All efforts must be made to eradicate the canker bacterium from infested areas. Citrus canker still does not exist in some countries or regions of countries where climatic conditions are favorable for pathogen establishment, which is probably because of rigid restrictions on the importation of propagating material and fruit from areas with canker. In the USA, quarantining areas affected by citrus canker is still practical. Eradication of infected and adjacent trees is the most effective means of protecting commercial citrus from the disease. Once positively identified, diseased trees in commercial groves are uprooted, placed in a pile, and burned. Surrounding, disease-free trees are destroyed as well, as an added precaution. In residential areas, diseased trees and surrounding, exposed trees are cut down or removed. Areas where trees have been destroyed must be kept free of citrus sprouts and seedlings. Movement of citrus fruit bud wood and other plant parts is prohibited to adjacent sites, where infected plants are located. All clothing, tools, and equipment used in infested areas must be properly disinfected (Gupta and Sharma 2008).

Cultural control

Raising canker-free nursery plants is the first essential step in citrus canker management. Where canker is endemic, certain cultural practices are used to reduce the severity of the disease. The infected plant parts should be pruned out and destroyed. Pruning infected shoots or plant parts during late summer and autumn can reduce the risk of infection the following spring. This is useful in reducing the inoculum density. Defoliation of canker-affected seedlings can also further reduce infection risk.

Disease-free nursery stock should be used. Numerous cases of new infections of citrus canker are linked to human and mechanical transmission. Humans can carry bacteria on their skin, clothing, gloves, hand tools, picking sacks and ladders. Vehicles can become contaminated by brushing against wet foliage or coming in contact with plant material. Machinery such as tractors, implements, sprayers and hedgers can similarly become contaminated and even inadvertently transport plant parts. In areas where citrus canker is resident, it is necessary to construct decontamination stations for personnel, vehicles and machinery which are sprayed with bactericidal compounds. It is imperative to avoid working in infected orchards when the trees are wet from dew or rain. The reduction of wind is another primary concern. Wind speeds are reduced by the deployment of windbreaks on the perimeter of the orchard or between the rows. Reduction of wind speed lowers the probability of

direct penetration of stomates by bacteria as well as entry of wind-induced injuries on foliage and fruit.

X. citri is the only plant pathogen to have been successfully eradicated in Mozambique, South Africa, New Zealand, and Australia and twice in the USA. Much success was achieved by implementing a policy of destroying infected trees and pruning all green wood on trees within 50 feet of the infected trees. The policy in Florida was changed in 1986, to cut and remove all "exposed" citrus trees within 125 feet of infected trees. Presently the citrus canker eradication programme in Florida (USA) has mandated the removal of infected citrus trees within a 1900 ft radius of an infected one. In the 5 years that this programme has been in effect in southeastern Florida, thousands of backyard citrus trees have been removed. Dooryard growers have the option of replacing trees which have been removed with either ornamental or tropical fruit trees. Many tropical fruit trees have the advantage of being both aesthetically attractive and producing delicious, nutritious fruit (Balerdi 2001).

The aqueous extracts of medicinal plants at 20% strength suppressed the growth of *X. axonopodis* pv. *citri*, *in vitro*. The plant extracts showing a high degree of suppression *in vitro* when evaluated *in vivo* by spraying over crop foliage using detached leaf inoculation technique, extracts of *Leucas indica* were the most effective in suppressing the disease and demonstrated 78.46 and 77.78% disease control compared with the control (Bora *et al*. 2001). Similarly, spraying with leaf extracts of *Tamarindus indica* resulted in the lowest citrus canker incidence (48%) under greenhouse conditions. Under field conditions, the number of diseased leaves and disease incidence was greatly reduced compared to the control after spraying of *T. indica* aqueous extracts (Leksomboon *et al*. 2001).

Chemical control

Prevention of primary infection on new shoots is one of the most important practices in the reduction of the disease. When environmental conditions are favourable for the spread of the disease, chemical control measures are not entirely effective. However, materials containing copper (Bordeaux mixture, copper hydroxide, basic copper chloride, copper oxychloride, and tribasic copper sulfate) are the most effective bacterial sprays for protecting leaves and fruits. These materials can reduce the incidence of disease, but they will not eliminate established infections. Extensive use of copper may also cause phytotoxicity problems in treated groves.

An application of Bordeaux mixture (4:4:50) or copper compounds (Cu content 50%) with $CaCO_3$ in late March has been recommended by Goto (1992) to reduce the disease. In addition to pruning, along with four sprays of copper oxychloride (0.5%) or Bordeaux mixture (1%) have been reported to be effective against the disease by Kishun and Chand (1987) under Indian conditions. Control of citrus canker with 4 sprays of copper oxychloride at 30-day intervals during the growth season was satisfactory (Verona *et al*. 2004). The copper treatments were not effective in controlling citrus canker at high inoculum concentration, however, at low inoculum concentrations, both Bordeaux mixture and copper oxychloride controlled the disease (Koller *et al*. 2006). A study was conducted to determine the sensitivity of *X. axonopodis* pv. *citri* strains from Parana, Brazil, to copper as well as to a mixture of copper with mancozeb. The highest copper concentration where bacterium grew was 50 µg/ml. However, 45.5% of the bacterial strains from orchards with regular sprays of copper compounds grew in the presence of 50 µg copper/ml. In contrast, only 13.4% of the strains from citrus orchards that never received copper sprays grew in such a copper concentration. Mixing mancozeb with copper increased the tolerance of bacterium to copper. Therefore, the recommendation of mancozeb mixed with copper for the control of the citrus canker bacterium should be reviewed (Meneguim 2007). Copper application significantly reduced damage to foliage and fruit, while windbreaks made little contribution to disease control (Behlau 2008).

Spraying 500X dilution of solution of 77% Kocide [copper hydroxide] wettable powder at 20-30 days after bud burst and spraying summer-autumn shoots at 10-15 days after bud burst could get 100% of the shoot leaves without canker infection (Zhong and Ling 2002; Pan 2004). Spraying with 500-fold and 400-fold solution of 77% copper hydroxide and 60% chlorothalonil solution, respectively, resulted in the efficient control of the disease (Fu and Xu 2001). $CaCl_2$ was applied to Kagzi Kalan lemon at 3 stages of fruit development (pea, marble and half-grown) at 4 concentrations (0.25, 0.50, 0.75, and 1.0%). $CaCl_2$ at 0.5% was the most significant at half-grown stage of fruit development in reducing fruit cracking (15.6%) with insignificant reduction in fruit weight and juice content, and increased yield tremendously (Sharma *et al.* 2002).

Streptomycin sulphate is specifically recommended against this disease and six sprays at 1000 ppm along with two prunings reduce the canker (Balaraman and Purshothman 1981). Foliar sprays of Streptocycline (100 ppm) plus copper oxychloride (0.1%) at 7 or 15 days interval have also been found effective in reducing the disease (Kale *et al*. 1994). While Zhang *et al*. (1996) observed best control of canker after foliar sprays of copper hydroxide (800 ppm). Gottawald and Timmer (1995) suggested use of windbreaks along with the application of copper bactericides as effective control measures of citrus canker. Integrated application of Bordeaux mixture or copper oxychloride, streptocycline and neem cake in combination with pruning during winter, budding stage and after petal fall was quite effective for controlling canker (Khodakaramian and Ghasemi 2002; Das and Singh 2003). However, for effective control of canker and gummosis of citrus, Jadeja *et al*. (2000) have suggested that the main trunk of the tree should be painted with Bordeaux paste and soil around the basal trunk be drenched with a mixture of metalaxyal + mancozeb or fosetyl AL. In addition foliar application of streptomycin sulphate + copper oxychloride be given three times a year i.e., before monsoon, in August and December. Control was also achieved by spraying 600×10^{-6} agrostreptomycin or 0.5% lime sulfur (calcium polysulfide) on young fruits and shoots by Ye *et al*. (2001).

Resistant varieties

In countries where the disease is well established and severe, only the more resistant types of citrus, such as 'Valencia' oranges and mandarins may be profitable. Seedless lime is reported to be resistant to citrus canker (Kishun and Chand 1987). Wei *et al*. (1995) have found 'Tangi' variety to be resistant to the canker in Japan. Some immune *Citrus* spp. have been reported to have narrow stomatal aperture, lower stomatal frequency (Pullaiah *et al*. 1994) and higher levels of phenols and amino acids (Pullaiah *et al*. 1993). Lesion number per inoculation site is sufficient for assessment of resistance of citrus genotypes to ACC without the necessity of conducting bacterial population assays. 'Lakeland' limequat is a promising seed parent for breeding acid citrus fruit that is resistant to ACC (Viloria *et al*. 2004). Citrus cultivar 'Setoka', obtained from a cross between 'Kuchinotsu No. 37' (Kiyomi × Encore No. 2) and 'Murcott', has been registered as 'Tangor Norin No. 8' in Japan and released in 1998 as a superior tangor [*Citrus sinensis* × *C. reticulata*] cultivar whose fruits ripen in February. Resistant to both citrus canker [*X. axonopodis* pv. *citri*] and citrus scab [*Elsinoe fawcettii*], this new cultivar has intermediate to weak tree vigour, strong parthenocarpic habit, tiny thorns, nearly seedless fruits with complete male sterility and polyembryonic seeds. Its oblate-shaped fruit weighs 200-280 g and has thin, orange to deep orange coloured rind, very tender and juicy flesh, pleasant and aromatic flavour, low acid content (0.8-1.2 g/100 ml) and high soluble solids (12-13%) concentration (Matsumoto *et al*. 2003). Late maturing cultivars 'Shiranuhi', 'Youkou' 'Miho-core' and 'Hareyaka' are

(Matsumoto *et al*. 1999a, 1999b, 1999c; Matsumoto 2001) resistant to citrus scab and canker; 'Amaka', derived from a cross between 'Kiyomi' tangor (*C. unshiu* × *C. sinensis*) is fairly resistant to citrus canker (Matsumoto *et al*. 2001). A mid and late maturing citrus cultivars 'Akemi' and 'Harumi' are resistant to citrus scab (*Elsinoe fawcettii*) and moderately resistant to citrus canker, which have been recommended for cultivation in Japan (Yoshida *et al*. 2000a, 2000b).

Introduction of resistance genes in susceptible cultivars is potentially the best procedure to control this disease. Protoplasts isolated from embryogenic callus of 'Newhall' navel orange, one of the leading commercial cultivars in China because of its seedlessness and other good qualities, 'Early Gold' sweet orange and 'Murcott' tangor were used for transformation. Plasmid DNA encoding the non-destructive selectable marker enhanced green fluorescent protein (*gfp*) gene (p524EGFP.1) and the plasmid DNA with a potential canker resistance gene (pC822) from the *Xa*21 gene family of rice (which provides broad spectrum *Xanthomonas* resistance in rice) were PEG-mediated co-transformed into protoplasts. Following protoplast culture in liquid medium and transfer to solid medium, transformed calli were identified via expression of *gfp*, physically separated from non-transformed tissue, and cultured on somatic embryogenesis induction medium. Transgenic embryoids expressing *gfp* were recovered. Shoots were regenerated from the three cultivars, and their growth was expedited by *in vitro* grafting. PCR analysis revealed that the *Xa*21 gene was present in all of the six analyzed shoots from 'Early Gold' sweet orange, and in none of the 19 analyzed samples of Newhall navel orange (Guo and Grosser 2004).

Induced systemic resistance compounds (ISRs), acibenzolar-S-methyl (Actigard), and harpin protein (Messenger) were assayed in the greenhouse against *X. axonopodis* pv. *citrumelo*, the cause of citrus bacterial spot (CBS), and *X. axonopodis* pv. *citri*, the cause of Asiatic citrus canker by applying as foliar sprays 3 to 7 days before inoculation reduced number of lesions when either bacterium at 10^3 or 10^4 CFU/ml was injection infiltrated into 'Swingle' citrumelo leaves. Based on this activity, the ISRs were evaluated in southern Brazil in orchards of sweet oranges with low to moderate canker disease incidence in spray programs with and without copper oxychloride (COC) and copper hydroxide (CuOH). Sprays of COC and CuOH were moderately to highly effective in reducing canker disease incidence and preventing premature fruit drop. Actigard or Messenger in combination with COC and CuOH, respectively, did not significantly reduce citrus canker incidence on foliage or fruit drop compared with Cu alone. The lack of additional control with ISRs means they cannot be recommended at this time to augment Cu programs for the management of citrus canker (Graham and Leite Jr. 2004).

Citrus rootstocks can exert some influence on fruit production and susceptibility of the plants to citrus canker. Reis *et al.* (2008) reported that the 'Swingle' citrumelo and 'Flying dragon' rootstocks induced the highest productivity index and, the lowest incidence of citrus canker disease on leaves and fruits. 'Rangpur' lime and 'Volkameriana' lime rootstocks, promoted a heavy crop load, however, showed higher susceptibility to citrus canker.

Biological control

The work on use of biocontrol agents against citrus canker is in a preliminary stage. Ota (1983) found a strain of *Pseudomonas syringae* antagonistic to *X. campestris* pv. *citri* which also prevented enlargement of lesions on infected leaves in citrus plants. While Pabitra *et al*. (1996) have observed *in vitro* inhibition of *X. campestris* pv. *citri* by *Bacillus subtilis*, *B. polymyxa*, *Pseudomonas fluorescens*, *Serratia marcescens*, *Aspergillus terreus*, *Trichoderma viride* and *T. harzianum* isolated from phylloplane of lemon. These were also observed effective in reducing the disease incidence when applied over crop foliage in the orchard. Akhtar *et al*. (1997) observed that diffusates (by using agar diffusion assay) of *Phyllanthus emblica*, *Acacia nilotica*, *Sapindus mukorossi* and *Terminalia chebula* inhibit the bacterium and exhibited an inhibition zone measuring 4.83-6.00 mm at 50 g/l. These diffusates (50, 20, 10 g/l) also reduce the number of lesions on detached leaves and fruits of grapefruit, thus exhibiting protective as well as curative actions. The crude extract of *Chebulic myrobalan* fruit at 50, 000 ppm spraying before inoculation and after that 3 times every 7 days, decreased wound sizes. Average wound size at 15, 20 and 30 days were 0.62, 0.97 and 1.40 mm while in the control treatment was 0.97, 1.84 and 3.00 mm, respectively (Vudhivanich 2008).

Integration of management practices

In countries where citrus canker is an established, ongoing problem, control of the disease is primarily achieved through a combination of tactics, including the production and use of disease-resistant plant varieties, use of protective sprays, and phytosanitary measures (use of certified nursery stock) and leafminer control. Outbreaks of citrus canker may also be reduced when windbreaks are constructed in windy areas with frequent applications of copper sprays. Copper sprays have been shown to reduce infection somewhat. Because the fruit is susceptible to canker during the first 90 days after petal fall, it is important to maintain a protective coating of a copper material on the fruit surface during this period. Two or three treatments may be needed for this purpose, depending on rainfall and cultivar susceptibility. Windbreaks can greatly reduce spread and severity of disease and increase the efficacy of copper sprays. Leafminer control is particularly important on young trees and certain cultivars that have a high proportion and greater frequency of vegetative growth flushes. To summarize, pruning of infected twigs along with sprays of Bordeaux mixture/copper is the best control measure. Complete protection of the plants is thus required throughout the season depending upon appearance of the disease. Streptomycin sulfate (1%) and garlic extract at (S), S/5 and S/10 restrict multiplication of *X. campestris* pv. *citri* [*X. axonopodis* pv. *citri*] whereas streptomycin sulfate (0.1%) + garlic extract (S) was superior in reducing the growth of the bacterium, followed by 0.1% + S/5 and 0.1% + S/10 combinations. The garlic extract was effective *in vitro*, but was not highly effective in greenhouse grown plants. However, its application at S/10 concentration along with 0.1% streptomycin sulfate reduced canker disease by 51.3% over the control, compared to a 60% reduction in the disease with the application of streptomycin sulfate at 1% (Khan *et al*. 2003).

FUTURE PERSPECTIVES

Bacterial canker of citrus is a serious disease worldwide. The pathogen has very wide host range and it is generally said that there is not even a single seedling/tree which is free of canker through out the world. The fruit infection phase of the disease is most damaging and cause of agony to the orchardists. The association of leaf minor has further aggravated the problem in the recent years which requires better understanding of interaction of leaf minor and bacterium and subsequent development of management strategies. Due to adaptability of the pathogen to all the species of Rotaceae, new strains have evolved whose pathogenic specialization and proper identification is still required to be established. More intense studies are needed for quick diagnosis and potential of phenylacetaldehyde *O*-methyloxime as diagnostic compound for bacterium is required to be thoroughly investigated. The disease once reported to be completely eradicated has again surfaced at its places of origin. This has questioned the validation of management technologies. There are no new chemicals which can be effectively utilized against bacterial diseases. Hence, other alternatives are required to be developed. Attention has to be paid to endophytes which may be useful in biotic man-

agement of the disease. A few resistant cultivars have been developed, however, induced systemic resistance can be the best option which need proper understanding and evaluation. Some progress has been made with respect to introduction of resistance genes in susceptible cultivars through molecular techniques which needs further strengthening and may be ray of hope in future for tackling this worldwide menace.

REFERENCES

Aiyappa KM (1958) Citrus canker - *Xanthomonas citri* (Hasse) Dowson. *Mysore Agriculture Journal* **13**, 164-167

Akhtar MA, Rahber Bhatti MH, Aslam M (1997) Antibacterial activity of plant diffusates against *Xanthomonas campestris* pv. *citri*. *International Journal of Pest Management* **43**, 149-153

Alvarez AM, Benedict AA, Mizumoto CY, Pollard LW, Civerolo EL (1991) Analysis of *Xanthomonas campestris* pv. *citri* and *X. campestris* pv. *citrumelo* with monoclonal antibodies. *Phytopathology* **81**, 857- 865

Bal RS, Dhiman JS (2005) An analysis of synchronous progress of canker-scab complex in Kinnow nursery. *Plant Disease Research* **20**, 147-150

Balaraman K, Purshothman R (1981) Control of citrus canker on acid lime. *South Indian Horticulture* **29**, 175-177

Balerdi C (2001) The tropical fruit garden after citrus canker eradication. *Proceedings of the Florida State Horticultural Society* **113**, 312-314

Balestra GM, Sechler A, Schuenzel E, Schaad NW (2008) First report of citrus canker caused by *Xanthomonas citri* in Somalia. *Plant Disease* **92**, 981

Behlau F, Belasque J Jr., Bergamin FA, Graham JH, Leite RP Jr., Gottwald TR (2008) Copper sprays and windbreaks for control of citrus canker on young orange trees in southern Brazil. *Crop Protection* **27**, 807-813

Belasque-Jr J, Parra PAL, Rodrigues NJ, Yamamoto PT, Chagas MCM, Parra JRP, Vinyard BT, Hartung JS (2005a) Adult citrus leafminers (*Phyllocnistis citrella*) are not efficient vectors for *Xanthomonas axonopodis* pv. *citri*. *Plant Disease* **89**, 590-594

Belasque-Jr J, Bassanezi RB, Sposito MB, Ribeiro LM, de Jesus JWC, Amorim L (2005b) Diagrammatic scales for citrus canker severity assessment. *Fitopatologia Brasileira* **30**, 387-393

Bock CH, Parker PE, Gottwald TR (2005) Effect of simulated wind-driven rain on duration and distance of dispersal of *Xanthomonas axonopodis* pv. *citri* from canker-infected citrus trees. *Plant Disease* **89**, 71-80

Bora LC, Das M, Samuel J (2001) Medicinal plant extracts for management of citrus canker and bacterial leaf spot of betelvine. *Journal of the Agricultural Science Society of North-East India* **14**, 159-164

Buragohain VP, Chand JN (1991) Variation among the isolates of *Xanthomonas campestris* pv. *citri* in Haryana. *Journal of Mycol*ogy *and Plant Pathology* **21**, 106

Chagas MCM, Parra JRP, Namekata T, Hartung JS, Yamamoto PT (2001) *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) and its relationship with the citrus canker bacterium *Xanthomonas axonopodis* pv *citri* in Brazil. *Neotropical Entomology* **30**, 55-59

Chakravarti BP, Porwal S, Rangarajan M (1966) Studies on citrus canker in Rajasthan. I. Disease incidence and survival of the Pathogen. *Labdev Journal of Science and Technology* **4**, 262-265

Chand JN, Pal V (1982) Citrus canker in India and its management. In: Raychaudhuri SP, Ahlawat YS (Eds) *Problems of Citrus Diseases in India,* Surabhi Printers and Publishers, New Delhi, pp 21-26

Chowdhury S (1951) Citrus Canker in Assam. *Plant Protection Bulletin* **3**, 78-79

Civerolo EL, Fan F (1982) *Xanthomonas campestris* pv. *citri* detection and identification by enzyme-linked immunosorbent assay. *Plant Disease* **66**, 231-226

Coletta-Filho HD, Takita MA, de Souza AA, Neto JR, Destefano SAL, Hartung JS, Machado MA (2005) Primers based on the *rpf* gene region provide improved detection of *Xanthomonas axonopodis* pv. *citri* in naturally and artificially infected citrus plants. *Journal of Applied Microbiology* **100**, 279-285

Das AK (2002) Pathogenic variability in *Xanthomonas axonopodis* pv. *citri*, causal agent of citrus canker. *Journal of Mycology and Plant Pathology* **32**, 247-248

Das AK (2003) Citrus canker - a review. *Journal of Applied Horticulture* **5**, 52-60

Das AK, Singh S (2003) Integration of chemicals and cultural practices for management of bacterial canker (*Xanthomonas axonopodis* pv. *citri*) in acid lime (*Citrus aurantifolia*). *Indian Journal of Agricultural Sciences* **73**, 570-571

da Silva ACR, Ferro JA, Reinach FC, Farah CS, Furlan LR, Quaggio RB, Monteiro VCB, vanSluys MA, Almeida NF, Alves LMC, doAmaral AM, Bertolini MC, Camargo LEA, Camarotte G, Cannavan F, Cardozo J, Chambergo F, Ciapina LP, Cicarelli RMB, Coutinho LL, Cursino SJR, El-Dorry H, Faria JB, Ferreira AJS, Ferreira RCC, Ferro MIT (2002) Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature* **6887**, 459-463

Doidge EM (1916) Citrus canker in South Africa. *South African Fruit Grow*er, August issue

Dunger G, Arabolaza AL, Gottig N, Orellano EG, Ottado J (2005) Participation of *Xanthomonas axonopodis* pv. *citri hrp* cluster in citrus canker and nonhost plant responses. *Plant Pathology* **54**, 781-788

Dunger G, Relling VM, Tondo ML, Barreras M, Ielpi L, Orellano EG, Ottado J (2007) Xanthan is not essential for pathogenicity in citrus canker but contributes to *Xanthomonas* epiphytic survival. *Archives of Microbiology* **188**, 127-135

Dye DW, Bradbury JF, Goto M, Hayword AC, Lelliot RA, Schroth MN (1980) International standards for naming pathovers for phytopathogenic bacteria and a list of pathover names and pathotype strains. *Review of Plant Pathology* **53**, 153-168

Falico de Alcaraz L (1980) Variability in *Xanthomonas citri* (Hasse) Dow. *Fitopatologia* **15**, 7-12

Fawcett HS, Jenkins AE (1933) Records of citrus canker from herbarium specimens of the genus *Citrus* in England and the United States. *Phytopathology* **23**, 820-824

Fu ZH, Xu ZH (2001) Study on the occurrence of citrus canker and its control. *South China Fruits* **30**, 15

Gabriel DW, Rolfe BG (1990) Working models of specific recognition in plant-microbe interactions. *Annual Review of Phytopathology* **28**, 365-391

Gabriel DW, De Feyter R (1992) RFLP analyses and gene tagging for bacterial identification and taxonomy. In: Gurr SJ, McPherson MJ, Bowles DJ (Eds) *Molecular Plant Pathology, A Practical Approach* (Vol I) IRL Press, Oxford, pp 51-66

Gabriel DW, Kingsley MT, Hunter JE, Gottwald TR (1989) Reinstatement of *Xanthomonas citri* (ex Hasse) and *X. phaseoli* (ex Smith) and reclassification of all *X. campestris* pv. *citri* strains. *International Journal of Systematic Bacteriology* **39**, 14-22

Garnsey SM, Ducharme EP, Lightfied JW, Seymour CP, Griffiths JT (1979) Citrus canker. *Citrus Industry* **60**, 5-6, 8, 10, 13

Golmohammadi M, Cubero J, Penalver JM, Quesada JM, Lopez MM, Llop P (2007) Diagnosis of *Xanthomonas axonopodis* pv. *citri*, causal agent of citrus canker, in commercial fruits by isolation and PCR-based methods. *Journal of Applied Microbiology* **103**, 2309-2315

Goto M (1992) Citrus canker. In: Kumar J, Chaube HS, Singh US, Mukhopadhyay AN (Eds) *Plant Diseases of International Importance (Vol III) Diseases of Fruit Crops*, New Jersey, Prentice Hall, Inc., pp 170-208

Goto M (1985) The role of extracellular polysaccharides of *Xanthomonas campestris* pv. *citri* in dissemination and infection: A review. *Abstracts on Fallen Leaf Conference on the Genus Xanthomonas*. Sept. 20-23, p 15

Goto M (1969) Studies on citrus canker in Japan. *Proceedings of the First International Citrus Symposium* **3**, 1251-1252

Goto M, Yaguchi Y, Hyodo H (1979) Ethylene production in citrus leaves infected with *Xanthomonas citri* and its relation to defoliation. *Physiological Plant Pathology* **16**, 343-350

Goto M, Takahashi T, Messina MA (1980) A comparative study of the strains of *Xanthomonas campestris* pv. *citri* isolated from citrus canker in Japan and cancrosis B in Argentina. *Annals of the Phytopathological Society of Japan* **46**, 329-338

Gottwald TR, Graham JH (2000) Canker. In: Timmer LW, Garnsey SM, Graham JH (Eds) *Compendium of Citrus Diseases* (2nd Edn), APS Press, St. Paul, pp 5-8

Gottwald TR, Timmer LW (1995) The efficacy of windbreaks in reducing the spread of citrus canker caused by *Xanthomonas campestris* pv. *citri*. *Tropical Agriculture* **72**, 194-201

Gottwald TR, Graham JH, Schubert TS (2002) Citrus canker: the pathogen and its impact. *Plant Health Progress* 1-32

Govinda Rao P (1954) Citrus diseases and their control in Andhra State. *Andhra Agriculture Journal* **1**, 187-192

Graham JH, Leite JRP (2004) Lack of control of citrus canker by induced systemic resistance compounds. *Plant Disease* **88**, 745-750

Guo WW, Grosser JW (2004) Transfer of a potential canker resistance gene into *Citrus* protoplasts using *gfp* as the selectable marker. *Acta Horticulturae* **632**, 255-258

Gupta VK, Sharma SK (2000) *Diseases of Fruit Crops*, Kalyani Publishers, Ludhiana, 344 pp

Gupta VK, Sharma SK (2008) *Diseases of Fruit Crops* (2nd Edn), Kalyani Publishers, Ludhiana, 290 pp

Hamlin SA (1967) Studies on occurrence of pathotypes in *Xanthomonas citri* (Hasse) Dowson. *Punjab Horticulture Journal* **7**, 90-93

Ishihara H, Uchida S, Masuda Y, Tamura K, Tsuyumu S (2004) Increase in telomerase activity in citrus inoculated with *Xanthomonas axonopodis* pv. *citri*. *Journal of General Plant Pathology* **70**, 218-220

Jadeja KB, Mayani NG, Patel VA, Ghodasara MT (2000) Chemical control of canker and gummosis of citrus in Gujarat. *Journal of Mycology and Plant Pathology* **30**, 87-88

Jesus-Jr WC, Belasque JJ, Amorim L, Christiano RSC, Parra JRP, Bergamin FA (2006) Injuries caused by citrus leafminer (*Phyllocnistis citrella*) exacerbate citrus canker (*Xanthomonas axonopodis* pv. *citri*) infection. *Fitopatologia Brasileira* **31**, 277-283

Kale KB, Kolte SO, Peshney NL (1994) Economics of chemical control of citrus canker caused by *Xanthomonas campestris* pv. *citri* under field conditions.

Indian Phytopathology **47**, 253-255

Khan LD, Hingorani MK (1970) Strain studies on *Xanthomonas citri* (Hasse) Dowson. *Journal of Horticultural Science* **45**, 15-17

Khan MA, Iqram F, Rashid A (2003) Comparative efficacy of garlic extract and streptomycin sulphate against *Xanthomonas campestris* pv. *citri* (Hasse) dye *in vitro*, and on the control of citrus canker in greenhouse. *Pakistan Journal of Botany* **35**, 987-991

Khan MA, Rashid A, Ikram F (2002) Weekly minimum temperature: one variable model to predict citrus canker disease. *Pakistan Journal of Phytopathology* **14**, 7-10

Khodakaramian G, Ghasemi A (2002) Effects of two antibactericidal chemicals and pruning on control of citrus bacterial canker disease in garden in south of Iran. *Seed and Plant* **18**, 316-327

Kishore V, Chand JN (1972) Citrus Canker in Haryana. *Haryana Agriculture University Journal of Research* **27**, 124-127

Kishore V, Chand JN (1975) Resistance of citrus to citrus canker caused by *Xanthomonas citri* – analysis of phenols and sugars. *Indian Phytopathology* **28**, 46-50

Kishore V, Chand JN (1972) Citrus canker in Haryana. *Haryana Agriculture University Journal of Research* **27**, 124-127

Kishun R, Chand R (1987) Studies on germplasm resistance and chemical control of citrus canker. *Indian Journal of Horticulture* **44**, 126-132

Koller OC, de Oliveira RBL, Nunes DS, Soglio FD, Panzenhagen NV, Sartori IA, Manteze F (2006) Chemical control of citrus canker in young plants managed in conventional and organic systems. *Ciência Rural* **36**, 1043-1048

Lee YH, Lee SD, Lee DH, Ji SH, Chang HY, Heu SG, Hyun JW, Ra DS, Park EW (2008) Differentiation of citrus bacterial canker strains in Korea by host range, rep-PCR fingerprinting and 16S rDNA analysis. *European Journal of Plant Pathology* **121**, 97-102

Leksomboon C, Thaveechai N, Kositratana W (2001) Potential of plant extracts for controlling citrus canker of lime. *Kasetsart Journal Natural Sciences* **35**, 392-396

Luthura JC, Sattar A (1940) Note on citrus canker (*Phytomonas citri*) in the Punjab. *Proceedings of the 27th Indian Science Congress Part III*, pp 228-229

Matsumoto R (2001) 'Shiranuhi', a late-maturing citrus cultivar. *Bulletin of the National Institute of Fruit Tree Science* **35**, 115-120

Matsumoto R, Okudai N, Yamamoto M, Takahara T, Yamada Y, Oiyama I, Ishiuchi D, Asada K, Ikemiya H, Murata H, Kuniga T, Shichijo T, Yoshinaga K, Uchihara S, Koizumi M, Iwanami T (1999a) A new citrus cultivar 'Youkou'. *Bulletin of the National Institute of Fruit Tree Science* **33**, 67-76

Matsumoto R, Okudai N, Yamamoto M, Yamada Y, Takahara T, Oiyama I, Ishiuchi D, Asada K, Ikemiya H, Murata H, Yoshinaga K, Uchihara S, Ieki H (1999b) A new citrus cultivar 'Miho-core'. *Bulletin of the National Institute of Fruit Tree Science* **33**, 57-66

Matsumoto R, Okudai N, Yamamoto M, Yamada Y, Takahara T, Oiyama I, Ishiuchi D, Asada K, Ikemiya H, Murata H, Yoshinaga K, Uchihara S, Ieki H, Shichijo T (1999c) A new citrus cultivar 'Hareyaka'. *Bulletin of the National Institute of Fruit Tree Science* **33**, 47-56

Matsumoto R, Yamamoto M, Kuniga T, Yoshioka T, Mitani N, Okudai N, Yamada Y, Asada K, Ikemiya H, Yoshinaga K, Uchihara S, Oiyama I, Murata H (2003) New citrus cultivar 'Setoka'. *Bulletin of the National Institute of Fruit Tree Science* **2**, 25-31

Matsumoto R, Yamamoto M, Okudai N, Takahara T, Yamada Y, Kuniga T, Oiyama I, Asada K, Ishiuchi D, Ikemiya H, Murata H, Uchihara S, Yoshinaga K, Ieki H, Iwanami T (2001) A new citrus cultivar 'Amaka'. *Bulletin of the National Institute of Fruit Tree Science* **35**, 47-56

Meneguim L, Rinaldi DAMF, Santos ACA, Rodrigues LS, Silva MRL, Canteri MG, Leite Jr. RP (2007) Sensibility of *Xanthomonas axonopodis* pv. *citri* to copper and mancozeb. *Fitopatologia Brasileira* **32**, 247-252

Nirvan RS (1960) Effect of antibiotic sprays on citrus canker. *Horticulture Advances* **4**, 155-160

Nirvan RS (1961) Citrus canker and its control. *Horticulture Advances* **5**, 171-175

Ota T (1983) Interaction *in vitro* and *in vivo* between *Xanthomonas campestris* pv. *citri* and antagonistic *Pseudomonas* sp. *Annals of the Phytopathological Society of Japan* **49**, 308

Pabitra K, Bora LC, Bhagabati KN, Kalita P (1996) Phylloplane microflora of citrus and their role in management of citrus canker. *Indian Phytopathology* **49**, 234-237

Padmanabhan D, Vidhyasekaran P, Rajagopalan CKS (1973) Physiology of citrus leaves infected by *Xanthomonas citri* (Hasse) Dowson with special reference to halo formation: respiration and oxidative enzymes. *Indian Journal of Experimental Biology* **11**, 359-361

Padmanabhan D, Vidhyasekaran P, Rajagopalan CKS (1974) Changes in photosynthesis and carbohydrates content in canker and halo regions in *Xanthomonas citri* infected citrus leaves. *Indian Phytopathology* **27**, 215-217

Pan XQ (2004) Experiment of control of citrus canker disease by Kocide fungicide. *China Fruits* **5**, 57

Parsai PS (1959) Citrus canker. *Proceedings of the Seminar on Diseases of Horticultural Plant*s, Simla, pp 91-95

Prasad MVR, Moses GJ Reddy GS (1978) Variability in *Xanthomonas citri,* the incitant of citrus canker. *Indian Phytopathology* **31**, 227-229

Prasad N (1959) Citrus canker. *Proceedings of the Seminar on Disease of Horticultural Plant*s, Simla, pp 87-88

Pria M, Christiano RCS, Furtado EL, Amorim L, Bergamin Filho A (2006) Effect of temperature and leaf wetness duration on infection of sweet oranges by Asiatic citrus canker. *Plant Pathology* **55**, 657-663

Pullaiah N, Moses GJ, Kumar PS (1994) Morphological nature of resistance in citrus canker disease (*Xanthomonas campestris* pv. *citri* (Hosse) Dye). *Journal of Research Andhra Pradesh Agricultural University* **22**, 139-140

Pullaiah N, Moses GJ, Kumar PSR (1993) Biochemical nature of resistance in citrus canker disease (*Xanthomonas campestris* pv. *citri* (Hasse) Dye). *Journal of Research Andhra Pradesh Agricultural University* **21**, 172-173

Reis B, Koller OC, Schwarz SF, Theisen S, Sartori IA, Nichele FS, Lorscheiter R, Petry HB (2008) Fruit production and incidence of citrus canker lesions on 'Monte Parnaso' navel orange budded on seven rootstocks. *Ciência Rural* **38**, 672-678

Ramakrishnan TS (1954) *Common diseases of citrus in Madras state*, Government of Madras Publication

Rangaswami G, Soumini RCK (1957) Disease of citrus canker in Madras State. *Indian Horticulture* **5**, 50-57

Rao YP, Hingorani MK (1963) Survival of *Xanthomonas citri* (Hasse) Dowson in leaves and soil. *Indian Phytopathology* **16**, 362-364

Rudolph K, Roy MA, Sasser M, Stead DE, Davis M, Swings J, Gossele F (1990) Isolation of bacteria. In: Klement Z, Rudolph K, Sands DC (Eds) *Methods in Phytobacteriology*, Akademiai Kiado, Budapest, pp 45-94

Saravanan L, Savithri P (2003) Relationship between citrus leafminer, *Phyllocnistis citrella* Stainton infestation and citrus canker, *Xanthomonas citri* (Hasse) Dawson incidence on acid lime. *Shashpa* **10**, 181-182

Schaad NW (1988) Initial identification of common genera. In: Schaad NW (Ed) *Plant Pathogenic Bacteria*, APS Press, St. Paul, pp 1-15

Schaad NW, Postnikova E, Lacy GH, Sechler A, Agarkova I, Stromberg PE, Stromberg VK, Vidaver AK (2005) Reclassification of *Xanthomonas campestris* pv. *citri* (ex Hasse 1915) Dye 1978 forms A, B/C/D, and E as *X. smithii* subsp. *citri* (ex Hasse) sp. nov. nom. rev. comb. nov., *X. fuscans* subsp. *aurantifolii* (ex Gabriel 1989) sp. nov. nom. rev. comb. nov., and *X. alfalfae* subsp. *citrumelo* (ex Riker and Jones) Gabriel *et al*. 1989 sp. nov. nom. rev. comb. nov.; *X. campestris* pv. *malvacearum* (ex Smith 1901) Dye 1978 as *X. smithii* subsp. *smithii* nov. comb. nov. nom. nov.; *X. campestris* pv. *alfalfae* (ex Riker and Jones, 1935) Dye 1978 as *X. alfalfae* subsp. *alfalfae* (ex Riker *et al*. 1935) sp. nov. nom. rev.; and "var. *fuscans*" of *X. campestris* pv. *phaseoli* (ex Smith, 1987) Dye 1978 as *X. fuscans* subsp. *fuscans* sp. nov. *Systematic and Applied Microbiology* **28**, 494-518

Schubert TS, Miller JW (2000) *Bacterial Citrus Canke*r, Gainesville, Florida, FDACS, Division of Plant Industry, 6 pp

Sharma RR, Saxena SK, Goswami AM, Shukla AK (2002) Effect of foliar application of calcium chloride on fruit cracking, yield and quality of Kagzi Kalan lemon. *Indian Journal of Horticulture* **59**, 145-149

Sleesman JP, Leben C (1978) Preserving phytopathogenic bacteria at -70°C or with silica gel. *Plant Disease Reporter* **62**, 910-913

Stall RE, Seymour CP (1983) Canker, a threat to citrus in the Gulf coast states. *Plant Disease* **67**, 581

Sun XA, Stall RE, Jones JB, Cubero J, Gottwald TR, Graham JH, Dixon WN, Schubert TS, Chaloux PH, Stromberg VK, Lacy GH, Sutton BD (2004) Detection and characterization of a new strain of citrus canker bacteria from Key/Mexican lime and alemow in South Florida. *Plant Disease* **88**, 1179-1188

Swarup S, De Feyter R, Brlansky RH, Gabriel DW (1991) A pathogenicity locus from *Xanthomonas citri* enables strains from several pathovars of *X. campestris* to elicit canker like lesions on citrus. *Phytopathology* **81**, 802-809

Swarup S, Yang Y, Kingsley MT, Gabriel DW (1992) A *Xanthomonas citri* pathogenicity gene, *pthA*, pleiotropically encodes gratuitous avirulence on nonhosts. *Molecular Plant-Microbe Interactions* **5**, 204-213

Takahashi T, Doke N (1984) A role of extracellular polysaccharides of *Xanthomonas campestris* pv. *citri* in bacterial adhesion to citrus leaf tissues in preinfectious stage. *Annals of the Phytopathological Society of Japan* **50**, 565-573

Traore YN, Bui TNL, Verniere C, Pruvost O (2008) First report of *Xanthomonas citri* pv. *citri* causing citrus canker in Mali. *Plant Disease* **92**, 977

Vauterin L, Swings J, Kersters K, Gillis M, Mew TW, Schroth MN, Palleroni NJ, Hildebrand DC, Stead DE, Civerolo EL, Hayward AC, Maraite H, Stall RE, Vidaver AK, Bradbury JF (1990) Towards an improved taxonomy of *Xanthomonas*. *International Journal of Sytematic Bacteriology* **40**, 312-316

Vauterin L, Hoste B, Kersters K, Swings J (1995) Reclassification of *Xanthomonas*. *International Journal of Systematic Bacteriology* **45**, 472-489

Venkatakrishnaiah NS (1957) Canker disease of sour lime and its control. *Journal of Mysore Horticultural Science* **2**, 40-44

Vernière CJ, Gottwald TR, Pruvost O (2003) Disease development and symptom expression of *Xanthomonas axonopodis* pv. *citri* in various citrus plant tissues. *Phytopathology* **93**, 832-843

Verona LAF, Theodoro G de F, Nesi CN (2004) Relationship between the frequency of copper oxychloride sprays and the incidence of citrus canker in sweet orange leaves. *Agropecuaria Catarinense* **17**, 64-66

Viloria Z, Drouillard DL, Graham JH, Grosser JW (2004) Screening triploid hybrids of 'Lakeland' limequat for resistance to citrus canker. *Plant Disease* **88**, 1056-1060

Vudhivanich S (2008) Decreasing symptom development of citrus canker by Thai herbal crude extract. *Proceedings of the 46th Kasetsart University Annual Conference*, 29 January – 1 February 2008, Kasetsart, pp 489-496

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Truper HG (1987) Report of the Ad-Hoc Committee on reconciliation of approaches to bacterial systematics. *International Journal of Systematic Bacteriology* **37**, 463-464

Wei JF, Wei LG, Qin GM, Luo GF (1995) Resistance of six recommended varieties of Japan navel orange to citrus bacteria. *Plant Protection* **2**, 18-19

Yang XJ, Chen FR, Xie SY (2002) Advances in research on the occurrence and control of citrus canker. *China Fruits* **5**, 46-50

Ye TM, Lin ML, Zeng R, Hu DS (2001) The occurrence of citrus canker on pummelo variety Guanximiyou and its control. *South China Fruits* **30**, 24

Yoshida T, Yamada Y, Nesumi H, Ueno I, Ito Y, Yoshioka T, Hidaka T, Ieki H, Shichijo T, Kihara T, Tominaga S (2000a) New citrus cultivar 'Harumi'. *Bulletin of the National Institute of Fruit Tree Science* **34**, 43-52

Yoshida T, Yamada Y, Ueno I, Shichijo T, Nesumi H, Hidaka T, Ito Y, Yoshioka T, Kihara T, Ieki H, Hirai M, Tominaga S (2000b) New citrus cultivar 'Akemi'. *Bulletin of the National Institute of Fruit Tree Science* **34**, 53-62

Zhang AJ, Hartung JS (2005) Phenylacetaldehyde O-methyloxime: a volatile compound produced by grapefruit leaves infected with the citrus canker pathogen, *Xanthomonas axonopodis* pv. *citri*. *Journal of Agricultural and Food Chemistry* **53**, 5134-5137

Zhang K, He S, Huang Z, Zhang K, He S, Huang Z (1996) Effect of several fungicides for control of citrus canker (*Xanthomonas citri* Hasse Dowson). *South China Fruits* **25**, 20

Zhong DZ, Ling NC (2002) Observation on the occurrence regulation of citrus canker disease and its control. *South China Fruits* **31**, 20-21