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Management of Citrus Canker in Argentina. A Review[*]

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ABSTRACT. Canker B was introduced in Argentina in 1927, it was restricted to a limited area and became extinct after the A type entered the same niche 50 years later. The A type entered the NE in 1974, the spread was fast from 1977 to 1980, slow and constant till 1990 when it became endemic. The presence of canker in the NW of Argentina was apparent in 2002, its introduction was undetectable by the methods used. This paper presents the current management of canker in Argentina and the system to export fresh fruits to EU markets. In NE Argentina canker intensity is the effect of the environment, the ENSO causes cyclic variations. Disease intensity is lowest with low spring rainfall. Economic loss is caused by quarantine restriction to fruit from canker-infested area imposed recently by some markets although fresh fruit may not be a source for spread. Production of healthy fruits in endemic areas it is possible knowing the dynamic of the disease. Populations of *Xanthomonas axonopodis* pv. *citri* (Xac) in lesion-less leaves and fruits of all citrus types are low in highly infected trees and undetectable in low intensity plants. An Integrated Plan to Reduce the Risk of Canker is underway since 1994; participation of growers is voluntary. Certification of selected, registered, symptoms-free plots, with permanent surveys requires: windbreaks around 2-4 ha; sanitation with disinfectants; application of copper containing products to young leaf- flushes and to developing fruits to prevent infection, and treatment of fruit in certified packing houses with SOPP at 2% for 2 min. and sodium hypochlorite at 0.02% for 45 sec to ensure elimination of any bacterial cell.

BACKGROUND AND OBJETIVES

In Argentina almost 54 million plants of citrus are grown, (58.9% are orange, 30.5% tangerine, 22.9% lemon, and 7.7% grapefruit) (Table 1). Total production is 2.6 million tons. The product is sold as fresh fruit and juice, oil and other in the internal market and for export (Table 2).

Quarantine restrictions on fresh fruit from canker-infected areas have been imposed since 1998 by the European Union, although introduction of canker to new areas has never been shown to

occur through infected or infested fruits. Furthermore, there is no data showing that treated citrus fruit carry viable *Xanthomonas axonopodis* pv. *citri* (Xac) or that infected fruit can start an epidemic (Canteros et al., 2001).

CITRUS CANKER IMPORTANCE AND WORLD DISTRIBUTION

Citrus canker. Citrus canker, caused by pathovars of *Xanthomonas axonopodis* (Hasse) Vaut. (Xa) (syn.: *X campestris* (Hasse) Dye has spread to new areas in the last decades. The importance of canker is based mainly in the quarantined condition of the causal agents, restrictions being imposed by canker-free countries that use exclusion as the control method. The disease exists since ancient times in Eastern Asia and is spreading to citrus growing countries for the last 40 years. A previous expansion of the disease occurred at the beginning of the twentieth century, when eradication campaings were successfull in USA, Australia, South Africa, and New Zealand, after several years of host elimination. Worldwide movement of people and products and change of environmental conditions might have made more difficult to eradicate the disease in modern times, except for isolated islands near Australia (Canteros 2001a, 2001b, 2001c, 2001d, 2001f, Stall and Civerolo, 1991, Stall et al, 1993).

Canker in Argentina. Canker exists in Argentina since 1928 (Canteros 2001b, 2001d, Canteros et al, 1985), Fawcett and Bitancourt, 1949). The B type was introduced around that time but its origin never could be traced. However, its appareance was coincidental with the worldwide expansion of canker at that time. The low agressivity and restricted host range confined the B type strains for 40 years to a small area and it dissappeared in 1978-90 after the introduction of the most aggressive A type in 1975 (Canteros et al, 1985). The A type of citrus canker, caused by *Xanthomonas axonopodis* pv. *citri* became endemic in northeastern (NE) Argentina after eradication efforts failed. Current management is by integrated methods with the production of for-export fruits in symptomsless plots (Canteros 2001c, 2000). Canker was absent in the NW of Argentina until recently., when its introduction and spread was evident after early-detection methods have failed.

THE HOST

Host range of citrus canker. All types of citrus are susceptible but extreme variation exists among species and cultivars. Among the economically important citrus types, grapefruit is very susceptible; lemon and some tangerines and some oranges can be affected to moderate degree, other oranges and tangerines are very resistant.

Susceptibility of leaves and fruits. Leaves of citrus are susceptible only for a short period at the moment of faster growth, at two to three weeks old (Stall et al, 1979, 1982). Adult citrus plants

produce leaf-flushes three to four times a year. Availability of susceptible tissue are high only in young trees, whereas it is reduced in production groves. Fruits of all cultivars are susceptible when young and become increasingly resistant with age, some getting very resistant at an earlier stage (Canteros, 1992). In infectivity titration experiments, a minimum of 10^3 cells per milliliter was necessary to get lesions on grapefruit fruits and only inoculum concentration of 10^6 give severe infection (more than 5 les/cm^2). A positive linear relationship exists between inoculum concentration and number of lesions per square centimeter (Canteros, 1992). The resistance in fruits is expressed, as in leaves (Stall et al, 1982), as lower number of lesions per unit area (Canteros,1992). When the amount of disease in leaves is reduced this will indirectly reduce the amount of disease in fruits. Knowing the period of susceptibility in fruits of the most economically important cultivars have improved the chance of controlling the disease.

THE PATHOGEN

Occurrence of citrus canker and the pathogen. Citrus canker exists since remote times in Asia and from there it was spread to other regions at the beginning, the mid and the end of last century (Canteros 2001d, Stall and Civerolo, 1991). Eradication was successful only after the first spread. The movement of people, and plants, and the climatic changes, makes it most difficult the eradication in modern times. Citrus canker is spreading in several places in the world. It was introduced recently to some Asiatic Arab countries in infected plants; it has reemerged in Florida (USA); and in Sao Paulo (Brazil) where eradication methods are by destruction of diseased and adjacent trees. Canker will continue to spread to other citrus production regions and the incidence and severity of the disease, after introduction, will vary according with the environment and the cultivars being planted (Canteros 2001a).

Forms or types of canker. When canker was recognized as a new disease by C. Hasse in USA, in 1915, no differences were described among strains (previously known as isolates). In the Southeast Asia only slight differences were described. However, with new epidemics starting at various places the concept of types or forms of canker have developed, starting at around 1949 (Fawcett and Bitancourt, 1949) when the canker in Argentina (now B type) was described as being very different from the most common Asiatic canker (now A type). The types are now important for quarantine restrictions and new variants are emerging in new epidemics. These variants may have existed for years in canker-endemic Asian countries on different citrus types that are now being introduced to other countries.

The types described, at present, are: **Canker A or Asiatic canker**. It is the most important and agressive form of canker, it existed from ancient times in Asia and have spread to several countries: Japan, Taiwan, China, India, France (Reunion Island), Argentina, Brazil; Paraguay, Uruguay, Bolivia,

Yemen, Saudi Arabia, Iran, Pakistan, USA (Florida) and others. It infects all types of citrus, as grapefruit, Key lime, lemon, orange, tangerine, tangor, and other hybrids. The causal agent is *Xanthomonas axonopodis* pv. *citri* (syn. = *X. campestris pv. citri*) (Stall et al, 1981 ; vauterin et al, 19995, 1991). **Canker B or South-American cancrosis**. It was described as false canker in 1927-29 in Argentina (provinces of Corrientes and Entre Rios). It was important only on fruits of lemon and the attacks were sporadics. After the introduction of the A type the B form has dissappeared and can not be isolated from the field any more. Now is mantained in Pathogen Collections only. At present, the bacterium was renamed *Xanthomonas axonopodis* pv. *aurantifolii* (syn= *X. campestris* pv. *aurantifolii*) (Canteros et al, 1985, Vauterin et al, 1991,1995). **Canker C or Key lime canker.** This type was described only in Brazil on Key lime, also known as limoneiro gallego (*C. aurantifolia*). Its occurrence is sporadic. Taxonomic studies of restricted numbers of strains of the causal agent named it as *Xanthomonas axonopodis* pv. *aurantifolii* (syn= *X. campestris* pv. *aurantifolii*). It may exists in other parts of the world where citrus it is not commercially important and thus description was never made (Stall and Civerolo, 1981, Stall et al, 1981). **Canker D or "Mexican bacteriosis ".** It was described as a new type of canker in Mexico, in 1981 when first appeared on Mexican lime (*C. aurantifolia*). Although several papers were published about these strains apparently isolated in Mexico, the existence of this type is now at least unknown, since the symptoms described previously as canker D are now recognized as caused by the fungus Alternaria. **Canker E, nursery canker or citrus bacterial spot.** This type appeared in Florida, USA, in 1984 on Swingle citrumelo. Eradication was attempted but was then stopped after research indicated that it was not a serious disease. The causal agent is *Xanthomonas axonopodis* pv. *citrumelo* (syn= *X. campestris* pv. *citrumelo*). The origin of this type was never clarified (Vauterin et al 1995, 1991). **Other types**. Some variations were observed among strains of some of the types described. Host range differences were found among some supposedly A type strains isolated in India and other strains from new epidemics in Arab countries. Research on pathogen variations are still needed (Canteros 2001f).

Characterization of natural populations of Xanthomonas causing canker in Argentina. Strains of *X. axonopodis,* isolated from canker lesions on citrus plants in NE Argentina in the last ten years were characterized. The A type is now the only type obtained in nature, type B strains were obtained from few lemon groves until 1979 in Corrientes and 1993 in Entre Ríos. After that time the strains dissappeared from the field.. The B strains are identified by the lack of growth in nutrient agar, lack of milk proteolysis, and lack of survival at 2 per cent NaCl. The A strains were positive in all those tests. One C type from Brazil was used por comparison. The B strains were consistently less agressive than the A strains in all citrus types (lemon, lime, orange, grapefruit, and tangerines). One C type was compared to A and B and it caused typical lesions only on Key lime and few small lesions in sour orange. Key lime was highly susceptible to all types (A, B, C). Growth experiments in grapefruit

and Key lime indicated that after leaf infiltration the A type continued to grow until 10 days in grapefruit and Key lime; the B and C types reached populations 100 times lower than the A in Key lime; and the C type stop growing after six days in grapefruit when the population (10^4-10^6 less than the A strain) started to decline sharply (Canteros and Naranjo, 2002).

Comparisons with other strains of different origin. Strains of *X*. *axonopodis* isolated from citrus plants in different parts of the world and present in the EEA INTA Bella Vista Collection were inoculated in several citrus varieties. The strains were first characterized as A, B, C, and unidentified types (Canteros et al, 1985, Stall et al, 1981). Nine A type strains isolated from different varieties at Bella Vista, Ctes., Argentina, were equally agressive in grapefruit or orange when low inoculum was infiltrated in leaves (25-50 lesions per sq cm), or inoculated high inoculum by pin-prick (2.5-3.5 cm les diam). After infiltration of six different citrus varieties, one A type strain gave 20-70 les per sq cm, and one B type gave 0.1-0.8 les in all plants, and one C type gave 2.8 les only in Key lime. When the infectivity in Key lime of four inoculum dosis for each A, B and C strains were log tranformed and linearized, the regression slopes of A and C were similar whereas the B type gave lowest numbers for all doses. Strains isolated from Key lime in India were compared with one A strain. Population growth and numbers were faster and higher for the Indian strains in Key lime than in grapefruit after eight days. The A strain reached higher populations than the Indian strains in both hosts, grapefruit and in Key lime (Naranjo and Canteros, 2002)

Molecular characterization of natural populations. In works initiated to characterize by molecular methods the canker-causing strains in Argentina with the final aim of developing appropiate diagnostic procedures, the REP-PCR method was tested. The ERIC, BOX, and REP universal primers were used, all strains were from the Collection of EEA INTA Bella Vista. Most strains were undistinguishabled by this method, although some of them showed one-band variation with one primer or another (Haelterman et al, 2002).

ECOLOGY

Disease cycle and ecology. The bacterium enters the young tissue of leaves, fruits, and twigs through stomates or injuries. It multiplies to form the classic corky lesion known as canker. Water is needed to emerge from lesions and to get to new susceptible tissue. Winddriven rain is thus necessary to move out and reinfect other tissue. Wind by itself is not important except when it causes injuries right before rain. New lesions are evident after 20 days in the field. To the untrained person symptoms will be visible only after 30-40 o even more days after the infection occurred. The most important source of inoculum are the lesions in a given tree. In NE Argentina infections are produced in young

fruits and leaves during rains in windy conditions (Canteros, 2001d).

Environment. Environmental conditions are very important in the intensity of canker in a given place. Studies should be conducted at different places to find the variations through the seasons and the years that will determine the expected severity (Canteros, 1998). Important factors are temperatures, relative humidity, and, most important, winddriven rain. The importance of the environment was well studied in Japan (Stall et al, 1993). Since the main inoculum come from the lesions in a given tree, the elimination of symptoms will help in decreasing the inoculum to avoid reinfectation with the rain.

Environmental conditions in Argentina. Through several studies it was determined that canker intensity will vary markedly in NE Argentina according with the variation of the predisposing factors even in plants without chemical sprays. The intensity in each tree and severity on fruits vary markedly with the seasons and are lowest on those years with low rainfall in the spring (Canteros, 1998). The weather causes cyclic variations, severity is lowest with low spring rainfall. The climatic conditions like those of the ENSO (El Niño- Southern Oscillation) are very important in the intensity and spread of canker and they are responsable for the cyclic variations. These variation are similar for all cultivars regardless of their resistance (Canteros, 1998) (Table 4). Inoculum increase markedly in ENSO seasons. Most of the inoculum come from lesions in a given tree. Spread by wind apparently is not very important in this area, except when exceptionally strong storms occurs wuch is highly improbable. Preliminary data obtained only twice during rainy days has found the bacterium no more than 30 meters than a infected tree (Miller et al, 1980, Stall et al, 1980, 1979). Further studies indicated that that is an excepcional occurrence since most Xac cells were found only under infected treeS and could not be detected few meters from them (Naranjo and Canteros, unpublished).

The environmental data were obtained at the Wheather Station EEA INTA Bella Vista, Corrientes, Northeast of Argentina, located at 28º 26' S; 58º 55' W; 70 m over sea level, with average annual rainfall of 1179.37 mm (SD: 275.2 mm), and a mean rainy days per year of 83 (SD: 12.5).

Populations of Xac in nature. Populations of Xac could be quantified by washing leaves and fruits and plating on semi-selective medium (lima bean agar, kasugamycin, yeast extract and cycloheximide) and by infiltrating susceptible leaves of grapefruit seedlings kept in growth chambers. Numbers detected ranged from undetected level on lesionless leaves and fruits of orange and lemon from sprayed low disease plots and 0 to 10^6 cells of Xac per leaf or fruit (mean: <10) from highly infected unsprayed plots of grapefruit, lemon and orange (Rybak and Canteros, 2001). Harvested lesionless fruits sampled from boxes containing mixed diseased and healthy fruits carried populations from 0 to 10^4 Xac cells per fruit (mean: 10^2) of all citrus before and after tratment by waxing with no

desinfestant. Both methods of detection are appropiate and could be used to quantify natural populations of pathogenic Xac (Rybak and Canteros, 2001).

Disease incidence and intensity. Disease incidence and intensity in each tree was monitored every 1-2 weeks in a scale 0-100%. Severity of disease on fruits was taken at midseason and at harvest using a three grades scale; 0= no symptoms; 1= one large or three small lesions; 2= more thyan one large or three small lesions per fruit. A formula was used to determine disease intensity. Disease intensity in fruits= {(% fruits Grade 0x0) + (% fruits G1x1) + (% fruits G2x2)/ (number of grades= 3)} (Canteros, 1998) (Table --).

Canker intensity can vary due to the increase of new healthy tissue or defoliation due to the disease. Variation also occurs with the seasons and the years. An inverse relationship exists among disease intensity in plants at midseason and percentage of healthy fruits at harvest, whereas a direct relationship occurs among intensity at midseason and at harvest. In years with low infection only grapefruit will differ from other cultivars in the severity of infection on fruits (Canteros, 1998) (Tables – and --).

Experimental groves. .Two experimental groves were used for disease quantification: one grove (without any for-canker spray) was planted in 1991, at a distance of seven meters among rows and lines, with the following citrus cultivars: Red Blush grapefruit (*C. paradisi* Macf.), Murcott tangor (*C. reticulata* Blanco x *C. sinensis*). Valencia orange (*C. sinensis* (L.) Osbeck), Eureka 22 lemon (*C. -limon* (L.) Burmf.), and Okitsu satsuma tangerine (*C. unshiu* Marc.), all of them grafted one-year-old plants. The design is complete randomized blocks, three replications (five plants per plot). Blocks were arranged according with the windbreak located to intercept the predominant winds occurring with rain. The grove was not sprayed with any bactericide. Fertilization was as commonly recommended and pests were managed with insecticides and acaricides.

The other grove (with for-canker spray) was planted in 1997 and 1998, at a distance of six meters among rows and four meters among lines for tangerines and six among rows and five among lines for others. Two blocks of 11 to 13 trees per replication were planted with 13 varieties/ rootstock; as follows: grapefruits Duncan/ Duncan, Foster/ rough lemon; lemon Eureka 22/ sour orange; lime (C limetticola) Tahití/ rough lemon; Tangerines Nova/ rangpour, Okitsu/ Rangpour; oranges Westin/ Rangpour; Newhall/ Rangpour, Salustiana/ Rangpour, Lane Late/ Rangpour, Valencia Late/Rangpour, Valencia Delta/ Citrange Troyer, Valencia Seedless/ rough lemon. Eucalypt trees were planted as windbreak all arounf the plot.

MANAGEMENT

Management of canker in Argentina. Canker B was managed without much problems for years. After the introduction of canker A strains it was necessary to start a new program (Falico and Canteros, 1978). Eradication efforts were in effect in 1977 when approximately 600.000 adult trees were destroyed. All nursery trees were destroyed and new planting stopped for several years. In Corrientes, only very infected trees were cut (200.000), mainly grapefruit. In Entre Ríos province, all affected (except lemon) plants were destroyed (400.000). In 1977-78, 2.500.000 trees were surveyed in Corrientes, 3900 plots in 541 properties. The percentage of plots of each variety infected with canker were: 89% of the lemon plots, 56% of grapefruit, 13% of orange, and 12% of the tangerines plots (Canteros, 2000, 2001c, Falico and Canteros, 1978). During the INTA-IFAS Cooperative Canker Project (1978-1983) timing of sprays was set and methods of research were developed. Resistance at the mesophyll level was found (Canteros 2001e, Miller et al, 1980, Stall et al, 1979). Proper application of copper containing bactericides gave good results for the management of the disease. Research on several aspects of the disease continued uninterrupted (Canteros, 2001c, 2001d, 2000, 1993).

Economic loss. When canker is severe, defoliation can occur, mostly in grapefruit, and this will debilitate the plant because of permanent growth if not measure is taken. Very infected fruits of susceptible varieties will also fall. Seedlings with canker are difficult to graft since a callus-like canker will develop in the injury made with the grafting-knife that will kill the new growth. However, the most important economic loss due to the disease is caused by the quarantine restrictions to the fruit from canker-infested area imposed by canker-free citrus growing countries (Canteros, 2000).

Production loss in Argentina. Total loss in productivity due to the disease is low, cost of sprays are not high compared to other citrus diseases and pests (Canteros, 2000, 2001c, 2001d). The effect also will vary considerably with the variety (Table --). When the A type was introduced in Argentina , and after several years of no new planting due to the spread of canker, planting was resumed with the advantage of the use of modern varieties. The production in symptoms-free plots are now possible to overcome the quarantine restrictions (Canteros et al 2001, INTA, 1997).

THE PROGRAM OF RISK MITIGATION FOR CITRUS CANKER AND ITS CAUSAL AGENT

Since 1990 a new program was started: The Program to Mitigate the Risk of Canker. This new program is based on more than 30 years results of research that provided the technology to obtain

fruits free of canker symptoms using the integrated management in certified traceable plots. This sistem was adopted after the most important market, the European Union, established some requirements for import of citrus from canker-affected countries (Canteros et al, 2001).

Traceability. Certification of symptomsless production plots. An Integrated Plan to Reduce the Risk of Canker is underway in NE Argentina since 1994, as part of the National Program of Citrus Health, being enforced by the National Plant Health Agency, Provincial Governments, citrus growers, and contribution of INTA (INTA 1997. Requirements to export fresh fruits includes certification of symptoms free plots. Participation of growers is voluntary (Canteros 2001d).

The European Comission Decision of 8 January 1998 Directive 98/83/EC states that fruits will be allowed into the EU in the following cases: "*Xanthomonas campestris* (all strains pathogenic to Citrus and relatives) … a) Country free of Xc; ... b) Area free of Xc;... c) Plots free of Xc at present growing season, symptoms free fruits collected from such plots treated with desinfestant in registered packing houses" (Canteros et al, 2001). To complay with these requirements, fruits are produced in NE Argentina in symptom-free plots in the "Program to Reduce the Risk of Canker" (the APPROACH System (INTA, 1997, Roberts et al., 1998)).

Certification of citrus production during the entire process from trees to export-box is made by SENASA, the animal and plant health Argentinian agency (http://www.sinavimo.gov.ar), complete details of the system can be found in this Internet site of the agency). The certification is based in the results of studies of the disease made in the country. Some of these information are presented in this review.

Symptoms and Diagnosis. First symptoms of canker are small, rounded, erumpent lesions in young leaves and twigs, and developing fruits, the color is light brown when they start but can change to several types when leaves become old and twigs harden. Most missidentification occurs when only old lesions are available. Windscar, insect damage, scab, and alternaria-leaf-spot can be easily confused with canker to the untrained eye.

Pathogen detection. The use of more than one method is highly desirable to avoid the occurrence of false positive or false negative. Serological and molecular methods can be used together with pathogenicity test. The infiltration of water suspension of crushed suspected lesions in susceptible leaves of grapefruit and Key lime to obtain lesions, and reisolation of the pathogen from those symptoms is until now the best diagnostic method . Other methods are being developed to use in quarantine facilities, such as tissue printing, which is easy, fast, economical, and suitable to

differentiate from other citrus blemishes and diseases of similar symptoms to avoid rejection of fruit due to false positives.

Fruit treatment for export. Certified packing houses should treat the fruit with approved desinfestants. The required treatments are inmertion during two minutes in sodium hipochlorite or one minute in SOPP or washing for 45 seconds with a formulation of soap-SOPP. Experiments were performed to test the efficacy of registered desinfectants to exclude any Xac epiphytic populations. Pathogen detection was by washing of fruits after treatment, plating on semi-selective medium, and infiltration of susceptible leaves of grapefruit and Key lime seedlings. Fruits and leaves with and without symptoms from diseased trees were used to ensure presence of Xac. Effect of desinfectants *in vitro* and on artificially infested fruits were determined. Commercial SOPP, OPP, and sodium hypochlorite (SH) were assayed at different rates and times; SOPP (2%, 2 min.) and SH (0,02%, 45 sec.) were the best treatments on fruits from infected plots, just in agreement with the currently required procedures. Both treatments together killed high concentrations of Xac when applied on fruit surface. The same products were highly effective against Xac *in vitro* even at very low rates (SOPP 0.03% and SH 0.001%) (Canteros et al, 2001).

Windbreaks. As was already stated winddriven rain is important in canker infection. In experiments to study the effect of the windbreak in canker intensity the Experimental grove was blocked according with the position of the windbreak located as to intercept the predominant wind accompaniyng the rains. Effect of the windbreak on disease intensity was assesed. The plants in each block were located at 19-47, 54-82, and 89-117 meters from the 25-m-tall *Casuarina* and *Grevillea* trees. Regression analysis among distance to the windbreak and disease intensity gave high positive correlation (R2: 0.62-0.96) throughout the study for all varieties. Disease intensity among blocks was statistically different ($p<0.01$). The difference in disease intensity among the first lane of plants (19 m from windbreak) and the last lane (117 m) was 2 to 10 times on different dates and for all varieties (Canteros, 1995a, 1998).

The windbreaks recommended are eucalipt, pine, Grevillea, Casuarina or any other tall trees. The effect should be to diminish the speed of wind, no to stop it completely. Windbreaks are now required around the 2-4 ha plot and even every 100 m in windy locations.

Leaf-miner and canker. The citrus leaf-miner (*Phyllocnistis citrella* Sta.) entered Argentina in 1966 and became widespread in very short time (Caceres, 2001, Canteros and Caceres, 2002). Soon was evident that the damage of the insect would be important for canker infection, as occurred for years in Southeast Asia. However, the occurrence of canker lesions on leaves with leaf miner damage

was evident only on infected trees whereas disease-free plants did not show new lesions, even those heavily damaged by the insect. Biological control or chemical sprays against the insect are recommended in for-export fruits. In plants where canker is severe, heavy defoliation will occur if the leaf-miner infestation is strong; after this sprouting will be abundant. Current research efforts are directed at finding the best treatment combination for both problems.

Sprays. Timing and products. All young citrus tissue should be treated to prevent infection. Sprays are applied to leaf- flushes in their susceptible stage (10 to 14 days old) and to developing fruits every 40 days. Recommended chemicals are copper products (tribasic coper sulfate, copper oxychloride, copper hidroxide, and copper oxide. Soluble powders are prefered over liquid forms. In Argentina, the most important sprays are those applied from bloom to four months later since they will prevent the increase of inoculum for the season**.**

Copper resistance. Timely application of copper sprays provided excellent control of the disease during 20 years. However, lack of control was noted in several groves and nurseries in 1994. A group of 580 strains collected from 1990 to 1993 and 67 strains obtained in 1994 from plots sprayed with copper and with poor canker control were grown in lima bean agar pH 7.0 with and without 200 ppm copper sulfate. Further confirmation of Cu^R was obtained in liquid media.Cu^S strains could not be recovered after 30 min in copper or copper + mancozeb, whereas in water or mancozeb alone they were alive after 36 h. CuR strains were still alive after 36 h except those with copper + mancozeb. The *BV5-4a* strains of Xa pv vesicatoria isolated in 1987 from tomato in Bella Vista was used as a positive control for copper resistance. Another group of 147 strains were obtained from a largest area in 1994, 1995, and 1996. None of the 580 strains grew in copper-ammended media whereas all 67 fresh isolated strains could grow after 48 h at 28ºC. Most of the Cu^R strains were from the same plots as several of the 580 strains obtained before 1994. Of the strains collected later, 56% were Cu^R whereas 44% were Cu^S (Canteros, 1999, 1994a, 1994b). Resistance to copper was demonstrated for the first time in the citrus canker organism. What was striking was the sudden widespread occurrence of the Cu^R strains troughout a large area since strains isolated prior to 1994 from the same groves were all Cu^S. Evidently, rapid selection and spread of the resistant strains occurred under the heavy pressure of numerous copper sprays applied in an attempt to control the high disease intensity. In Florida, susceptible strains of Xcv were isolated even after several years of the first appareance of resistant strains (Canteros 1990, Canteros et al., 1995, 1991, 1990, Pohoronezny et al., 1992). In this part of Argentina the citrus, tomato and pepper growing areas overlap. Further studies demonstrated that the resistance is plasmid encoded in Xac as in Xav encoded and that hybridization occurs among the Cu^R encoded DNAfrom Xav and Xac (Canteros, unpliblished).

Control of copper-resistant strains. Data obtained during ten years of spray trials on grapefruit seedlings infected with Cu-susceptible or Cu-resistant strains were used. Addition of mancozeb to copper products eliminated all Xac cells in water after 15 min. and gave excellent control of canker in the field. Other mixtures of Cu products, as addition of ferric sulfate were effective but toxic, and a product based on inorganic Cu and organic cation was effective only when mixed with 0.3 per cent of Cu compounds containing at least 50 percent metallic Cu. Copper plus mancozeb are now being used routinely in groves affected by canker caused by Cu resistant strains of Xac whereas Cu compounds alone still can be used in plants infected by Cu susceptible strains (Canteros 2002, 1995b).

Selective localized pruning. A method used for years in Japan in canker infected plants is the pruning of affected tissue in Fall and/or Winter. This will decrease the available inoculum very sharply. Pruning of affected tissue is used in Argentina in new planting and in plots treated to get canker-free fruits. The objective is to eliminate all diseased tissue in selected plots. Herbicide defoliation is recommended in heavily infected plots to start a program toward the objective of keeping it free of canker symptoms.

Sanitation. Use of desinfestant are recommended in selected plots. All equipment used in the plot should be desinfested. Hands, clothing and gloves of laborers, collecting boxes and any other tools should be treated. Cuaternary ammonium, fosforic acid-iodine solutions, sodium hypochlrite or 70% ethanol can be used

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Only the experimental evidence was and will continue to be necessary for the successful management of this disease in endemic regions and in the new regions where it could be introduced.

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Table 1. Citrus trees in different regions, provinces, and for different species in Argentina in the year 2002. Numbers are expressed in 1000 X.

Regions	Lemon	Orange	Tangerine	Grapefruit	Citrus Total
NE	2191.5	17098.0	14882.1	1172.3	35344.7
Misiones	450.0	977.4	1480.5	128.3	30036.2
Corrientes	1016.5	6800.0	3905.0	153.0	11874.5
Entre Ríos	595.0	7140.0	9120.0	400.0	17255.0
Formosa	54.9	46.0	40.0	328.8	469.6
Chaco	12.6	28.0	15.5	124.8	180.9
Buenos Aires	62.6	2107.4	321.1	37.5	2528.6
NW	10144.7	3860.4	1534.3	2980.5	18519.9
Jujuy	494.5	1092.5	496.1	195.0	2278.1
Salta	577.2	1499.9	240.2	2646.0	4963.3
Tucumán	9045.0	768.0	168.0	115.0	10096.0
Catamarca	28.0	500.0	630.0	24.5	1182.5
Country Total	12336.2	20959.1	16416.4	4152.8	53864.6
Percent (%)	22.9	38.9	30.5	7.7	100.0

Source: FEDERCITRUS-INTA Informes Citrícolas 2002. Bs As, 47 p.

Table 2. Total production of citrus in Argentina, amount and percent presented for each variety and final destination: fresh fruit or processing (juice, essential oil, others), and end market: national or for export. Values are expressed in 1000 X tons.

	Argentina market						Export				
Citrus type	Production	%	Fresh fruit	%	Processing	%	Fresh fruit	%	Processing	%	Consumption (kg/person/year)
Orange	618.7	23.9	426.3	68.9	31.2	5.0	23.7	3.8	137.5	22.2	11.77
Tangerine	457.0	17.6	374.8	82.0	9.4	2.1	41.4	9.1	31.4	6.9	9.18
Grapefruit	215.9	8.3	69.4	32.2	11.9	5.5	82.0	38.0	52.5	24.3	1.98
Lemon	1299.7	50.2	47.6	3.7	182.1	14.0	268.1	20.6	802.0	61.7	1.93
Total	2591.2	100	918.1	35.4	234.6	9.1	415.2	16.0	1023.4	39.5	24.86

Source: FEDERCITRUS-INTA Informes Citrícolas 2002. Bs As, 47 p.

Table 3. Total and average rainfall at periods of highest values (seven selected consecutives months) related to the ENSO and means of 1948-97 period. Agrometeorological Station EEA INTA at Bella Vista, Corrientes, Argentina. Location: 28º 26' S; 58º 55' W; 70 m over sea level.

Years		1958/	1965/	1972/	1976/	1982/	1986/	1991/	1997/	2002/
Month	(1948-1997) Mean	1959	1966	1973	1977	1983	1987	1992	1998	2003
Oct	143.5	12.0	241.4	255.3	94.0	134.0	151.3	105.9	267.3	244.0
Nov	130.4	150.2	174.6	186.2	59.4	49.0	229.1	169.6	162.0	212.0
Dic	130.9	273.6	239.7	143.2	223.6	42.9	142.2	190.0	265.3	329.0
Jan	126.7	296.0	240.5	269.2	242.1	321.4	190.8	107.5	259.9	110.2
Feb	142.2	482.0	174.9	71.8	139.8	178.4	146.8	187.7	206.7	146.8
Mar	152.8	185.0	205.5	318.1	181.4	233.9	160.2	252.7	371.4	247.5
Apr	139.6	84.7	174.0	207.2	174.7	129.0	206.2	427.7	394.8	158.0
May	76.21	195.5	86.0	62.5	39.5	87.9	72.2	123.7	44.9	12.0
Total (7mo)	966.0	1667.0	1450.6	1451.0	1115.0	1088.6	1226.6	1458.9	1927.4	1447.5
Mean/ month	138.0	238.1	207.2	207.3	159.3	155.5	175.2	208.4	275.3	206.8

Table 4. Disease intensity (DI) determined in plants of different varieties of the experimental grove with no-spray for canker, at different weeks after planting of the trees. DI= intensity (%); W= weeks after planting. Numbers followed by the same letter (in each column) are not different by ANOVA and Duncan MRT ($p<0.01$ or 0.05).

CULTIVAR	*19Dic 1991* W:16	*11Jun 1992* W: 41	*23Dic 1992* W: 69	*20Aug 1993* W:103	*30Dic 1993* W:122	*15Jun 1994* W:146	*11Jan 1995* W:176
Grapefruit	5.33	38.07	58.33 a	93.3 a	43.0a	39.9 a	24.27[a]
Lemon	0	4.93	11.97b	7.46c	4.67c	1.13c	2.4b
Orange	0	4.67	9.07b	17.3c	6.93c	4.0c	1.27b
Murcott tangor	0	13.07	19.53b	42.0b	22.7b	9.3b	2.67b
Satsuma tangerine	0	0.80	5.07b	6.2c	1.13c	0.4c	0b
Mean	1.07	12.31	20.79	33.25	15.69	10.95	6.12

CULTIVAR	*18May 1995* W:194	*3Jan 1996* W:227	*17Jun 1996* W:251	*27Dic 1996* W: 278	*6Jan 1997* W:280	*20Jun 1997* W:304
Grapefruit	49.8 a	3.2[a]	34.73a	27.07a	40.0 a	39.67a
Lemon	4.33b	0.27a	7.33b	7.47b	3.4b	3.40b
Orange	4.2b	0.33a	5.27b	12.53ab	5.8b	6.33b
Murcott tangor	14.8b	3.07a	13.1ab	6.13b	8.07b	7.73b
Satsuma tangerine	1.73b	0a	2.4b	0.27b	0.8b	0.60b
Mean	14.97	1.37	12.56	10.69	11.61	11.47

CULTIVAR	*30Dic 1997* W:333	*29May 1998* W:352	*4Jan 1999* W:382	*9Jun 1999* W:404	*10Dic 1999* W:430	*23May 2000* W:455
Grapefruit	67.33a	60.67[a]	31.00a	40.67	16.33	27.33
Lemon	21.67b	27.67b	6.33c	10.53	12.0	4.07
Orange	32.80b	37.00ab	10.07b	14.33	13.20	8.47
Murcott tangor	34.47b	39.67ab	10.67b	19.33	14.33	6.13
Satsuma tangerine	4.40b	22.47b	3.2c	4.87	3.47	0.67
Mean	32.13	37.49	12.25	17.95	11.87	9.33

CULTIVAR	*7Dec 2001* W:535	*4 Jan 2002* W:539	*22Mar 2002* W:550	*19Feb 2003* W:598	*6Nov 2003* W:635	*21Jan 2004* W:646
Grapefruit	52.33	43.00	34.00	42.00	24.33	63.7
Lemon	8.47	8.53	2.40	15.40	4.70	11.50
Orange	11.8	11.02	4.87	14.25	8.00	16.80
Murcott tangor	7.06	12.47	3.33	11.53	4.40	19.10
Satsuma tangerine	0	1.33	0 c	2.26	1.13	2.00
Mean	15.93	15.27	8.92	17.09	8.50	22.60

Table 5. Disease intensity determined in fruits (three grades formula) of different varieties without canker sprays at different weeks after planting. Numbers followed by the same letter, in each column, are not different by ANOVA and Duncan RMT (p< 0,01 or 0,05).

CULTIVAR	*9May95* W: 193	*16May96* W: 246	*15Mar97* W: 289	*15Mar97* W: 289	*1997-98* W: 352	*4Jan99* W: 383
Grapefruit	46.16[a]	36.29a	45.71a	45.71a	67.22	50.09a
Lemon	6.97 b	8.89b	9.33b	9.33b	29.27	6.00 b
Orange	5.71 b	8.45b	10.22b	10.22b	26.43	10.44 b
Murcott tangor	4.13 b	12.45b	11.97b	11.97b	35.26	8.22 b
Satsuma tangerina	0 b	0 b	0 b	0 b	0*	0 c
Mean	12.6	13.22	15.45	15.45	31.64	14.95

CULTIVAR	*4Jan99* W: 383	*23May00* W: 455	*30Mar01* W: 499	*17Jul02* W:567	*19Feb03* W: 598	*21Jun04* W: 646
Grapefruit	50.09a	23.00	45.17	30.04	54.75	53.07
Lemon	6.00 b	5.30	1.78	5.11	22.50	14.47
Orange	10.44 b	2.20	6.67	3.56	10.94	14.90
Murcott tangor	8.22 b	1.52	4.22	3.63	17.60	23.13
Satsuma tangerine	0 c	0	0	0	0	0
Mean	14.95	6.60	11.60	8.47	21.16	21.11