Towards an improvement of citrus canker control in Reunion island

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ABSTRACT

HISTORY OF CITRUS CANKER IN REUNION. Citrus industry in Reunion started in the 1960's, with the introduction of propagating plant material from countries where citrus bacterial canker disease (CBCD) had never been reported. CBCD possibily occurred in Reunion at that time on wild citrus trees in Creole gardens. Control of CBCD in nurseries can potentially improve disease control in new grove planting, as infected plants would be the main source of primary inoculum. **DEVELOPMENT OF DIAGNOSTIC TOOLS.** *Xanthomonas axonopodis* pv *citri* (*Xac*), associated with infected nursery citrus, is a target of international phytosanitary quarantine. A sensitive and specific detection technique of *Xac* was developed allowing detection of approximately 10^2 cells per gram of citrus leaf. **CLIMATIC CONDITIONS IN REUNION AND SPREAD OF *XAC* IN GROVES AND NURSERIES.** In most citrus growing areas in Reunion, year-round temperatures and annual rainfall are conducive to infection by *Xac*. **SPATIOTEMPORAL ANALYSES OF CBCD IN SIMULATED NURSERIES.** Spatial and spatio-temporal studies confirmed that disease patterns were aggregated in the field over time. Increase of disease rates was greater in plots with overhead irrigation, and in the case of plots with drip irrigation, it was associated with natural rainfall. To minimize CBCD transmission to a new grove, a modernization scheme for local citrus plant production was proposed. **DURABILITY OF A CLEAN PLANTING STOCK STRATEGY.** Grapefruit plants produced according to this improved scheme have been planted under various environmental conditions to experimentally determine the durability of citrus canker control resulting from the use of disease-free plants combined with other integrated control measures. **CONCLUSION** The improvement of the citrus nursery production scheme will essentially benefit citrus cultivars of low or moderate susceptibility to CBCD.

KEYWORDS
Réunion, *Citrus*, épidemiology, diagnostic, *Xanthomonas axonopodis* pv *citri*.

Vers une amélioration du contrôle du chancre bactérien des agrumes à l'île de la Réunion.

RÉSUMÉ

HISTORIQUE DE LA MALADIE À L'ÎLE DE LA RÉUNION. Les plantations industrielles d'agrumes à la Réunion, créées avec des plants venant de pays sans chancre bactérien des agrumes (CBA), datent des années soixante. Cependant, il se peut que cette maladie ait été alors présente à la Réunion sur certains arbres de jardins créoles. Le contrôle du CBA en pépinières pourrait permettre d'améliorer les conditions sanitaires des nouvelles plantations. **DÉVELOPPEMENT D'OUTILS DE DIAGNOSTIC.** Comme *Xanthomonas axonopodis* pv *citri* (*Xac*), disséminé par les jeunes agrumes contaminés en pépinières, fait l'objet de mesures internationales de quarantaine, une technique de détection spécifique et sensible de cette bactérie a été développée. Elle permet de détecter l'agent pathogène dès, environ, 10^2 cellules/g de tissus foliaire. **CONDITIONS CLIMATIQUES DE L'ÎLE ET DISSÉMINATION DE *XAC* EN VERGERS ET PÉPINIÈRES.** Dans presque toute l'île, les températures et les hauteurs de pluies sont toute l'année favorables à l'infection des plants par *Xac*. **ANALYSES SPATIOTEMPORELLES DE LA PRÉSENCE DU CBA EN PÉPINIÈRES SIMULÉES.** Des analyses spatiotemporelles de l'évolution de la maladie ont mis en évidence une distribution agrégative de l'inoculum. Cette évolution est plus accentuée dans les parcelles irriguées par aspersion et associée aux chutes de pluies naturelles dans les parcelles irriguées au goutte à goutte. Pour minimiser la transmission de la maladie lors de nouvelles plantations, un schéma amélioré de production locale de plants a été élaboré. **PERENNITÉ DE LA STRATÉGIE DE PLANTATION DE MATÉRIEL SAIN.** Des pomelos obtenus par ce schéma ont été plantés sous diverses conditions expérimentales. La plantation de matériel sain a été accompagnée d'autres mesures de lutte intégrée. **CONCLUSION.** Le schéma élaboré n'est valable que pour la plantation d'agrumes peu ou moyennement sensibles au CBA.

MOTS CLÉS
Réunion, *Citrus*, épidémiologie, diagnostique, *Xanthomonas axonopodis* pv *citri*.

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introduction

Asiatic citrus canker, caused by *Xanthomonas axonopodis* pv *citri* (*Xac*), is a devastating disease of some citrus species in areas in which high temperatures and rainfall occur at the same time of the year. *Xac* is endemic to many countries of Asia, the Indian subcontinent, South America, and has recently been discovered in Florida (GOTTWALD et al, 1997).

history of citrus canker in Reunion

The first record of citrus bacterial canker disease (CBCD) in Reunion was by BRUN (1971). However, it is likely that CBCD occurred long before that time. The disease was reported in neighboring islands of the Mascarene archipelago in the 1940's (WIEHE, 1941). The first attempts to develop a citrus industry in Reunion started in the 1960's, with the introduction of propagating plant material which originated exclusively from countries where CBCD had never been reported (AUBERT, 1985). It is possible that CBCD had infected wild citrus trees in 'Creole gardens' and that it spread to newly established groves in the first decade following development of citrus industry in the island.

However, citrus greening, associated with *Liberobacter asiaticum* and *L africanum* (JAGOUEIX et al, 1994), was considered a more serious constraint to citrus production than CBCD. As a consequence, the first research efforts dealt with citrus greening, which was controlled by the biological control of its vectors *Diaphorina citri* and *Trioza erytreae* (AUBERT, 1985). The control of citrus greening resulted in an increase in citrus surfaces which consisted primarily of mandarins and mandarin hybrids which are tolerant to CBCD. CBCD is now endemic throughout the island. There is presently a need to increase the diversity of commercial citrus cultivars in Reunion. Therefore, CBCD control will likely become an issue, particularly for more susceptible cultivars.

Traditionally, nurserymen in Reunion grow citrus in polyethylene plastic bags with overhead irrigation. Infected nursery plants are believed to be the main source of primary inoculum in newly established citrus plantings (VERNIÈRE, 1992). Therefore, control of CBCD in nurseries can potentially improve disease control in new planting. The Asiatic citrus leaf miner (*Phyllocnistis citrella*) was introduced in Reunion in February 1995 (QUILICI et al, 1995) and has further complicated the control of CBCD because its feeding galleries expose susceptible leaf mesophyll tissues to bacterial infection.

development of diagnostic tools

Given that the spread of *Xanthomonas axonopodis* pv *citri* (*Xac*) is associated with movement of infected nursery citrus plants in most countries where CBCD is endemic, *Xac* is a target of international phytosanitary quarantine. Therefore, sensitive and specific detection techniques for *Xac* are needed to support quarantine efforts. Polymerase chain reaction(PCR)-based methods for CBCD detection were developed via a cooperative research program between Cirad (Centre de coopération internationale en recherche agronomique pour le développement, France) and USDA/ARS (United States Department of Agriculture, Agricultural Research Service, United States).

A plasmid DNA fragment that is present in all assayed strains of *Xac* was cloned (PRUVOST et al, 1992). This fragment was absent from the genome of all of the citrus saprophytic bacteria tested and of most plant pathogenic *Xanthomonas* (except strains of pathovars *vignicola*, a pathogen of several leguminous plants, and a single strain of pv *bilvae*), including *X axonopodis* pv *citrumelo*, the causal agent of citrus bacterial spot in Florida (HARTUNG, 1992). The nucleotide sequence of this DNA fragment was determined (HARTUNG et al, 1993) and an immunocapture nested PCR protocol (IC-N-PCR) associated with colorimetric detection of amplified DNAs was optimized (HARTUNG et al, 1996). This technique enables the detection of a single template molecule or bacterial cell per reaction (± 10^2 cells per gram of tissue). Therefore, it is approximately

100 times more sensitive than immunofluorescence stain techniques and 10 000 times more sensitive than ELISA-based methods. Nested PCR protocols allow a high sensitivity, but are sometimes subject to problems of false positive results associated with DNA carry-over (KWOK, 1990). The latest improvement of the technique consists of a single tube nested PCR procedure (PRUVOST and HARTUNG, unpublished data) adapted from the results of SELLNER et al (1994).

climatic conditions in Reunion and spread of *Xac* in groves and nurseries

Xac can be disseminated through grove managment, movement of infected plant material, and meteorological events associating wind and rain (GOTO, 1992). Although some reports indicate that short distance spread of *Xac* by rain splash can occur within citrus nurseries planted under high densities (SERIZAWA et al, 1969; GOTTWALD et al, 1988), movement from tree to tree by climatic events in orchards is mostly associated with wind-driven rains which transport inoculum and result in stomatal flooding by *Xac*. Winds of 6.5 m/s or above associated with rains are known to spread *Xac* (SERIZAWA et al, 1969; SERIZAWA and INOUE, 1975; KUHARA, 1978; SERIZAWA, 1981; GOTTWALD et al, 1992a; GRAHAM et al, 1994; KOIZUMI et al, 1996). Climatic data analyses for several sites over a period of several years in Reunion indicated that these conditions are mostly associated with occurrence of tropical depressions or hurricanes.

There are three main climatic seasons in Reunion:
– from May to September, cool and dry weather prevails;
– temperatures rises from October and the weather remains mostly dry until December;
– hot and humid weather occurs from January to April.

Hurricanes occur during the latter season and a rapid increase of Asiatic canker is, as in many tropical areas, associated with the passage of hurricanes over the island. In most citrus growing areas in Reunion, year-round temperatures are conducive to infection by *Xac*. In Japan, CBCD is usually severe in areas with annual rainfall exceeding 1 500 mm, and mild when annual rainfall is less than 1 000 mm or monthly rainfall is less than 100 mm (KOIZUMI, 1985). In Reunion, annual rainfall of more than 1 500 mm occur in most citrus growing areas, with the exception of sites located to the West and South-West of the island at less than 300–500 m altitude.

spatiotemporal analyses of CBCD in simulated nurseries

Most data dealing with epidemiology of CBCD were obtained by American and Japanese scientists either in Florida, Argentina, or Japan (GOTO, 1992; STALL et al, 1993). These countries are characterized by the occurrence of a marked winter season, and are quite different from tropical areas where CBCD can develop year-round. Therefore, it is important to study CBCD epidemics in tropical countries where infection and inoculum production is continuous (CIVEROLO, 1994).

We studied CBCD development on Mexican lime (*Citrus aurantifolia*) in simulated nurseries established in June 1994 at Cirad in Saint-Pierre (altitude 150 m, annual average rainfall 900 mm). The influence of various irrigation systems was evaluated by comparing CBCD development on plots with overhead irrigation, mist irrigation, and drip irrigation. Source plants inoculated with *Xac* were randomly placed in the plots, so that 5% of plants in each experimental plot were initially diseased (disease incidence DI=0.05). Disease ratings were performed weekly until December 1994 on each plant in the plots.

At the end of the experiment, disease incidence ranged from: 0.36–0.49, 0.10–0.21, and 0.12–25 in plots with overhead irrigation, mist irrigation, and drip irrigation, respectively. Linear, exponential, and Gompertz models were fitted to the data by linear

regression analysis to identify the superior model. Because the exponential model was the most appropriate by linear regression, a more rigorous nonlinear exponential model was then used. The appropriateness of the exponential model was assessed by examining the residual plots and by correlation analysis of observed versus predicted values. Rates of disease increase were compared by *t*-tests to evaluate the influence of irrigation systems on disease development. Disease development in plots with overhead irrigation was statistically more rapid than in plots with either mist or drip irrigation. The main increase of disease incidence in plots with mist or drip irrigation was associated with the occurrence of natural rainfall.

As it has been shown for CBCD development in nurseries in Argentina (GOTTWALD et al, 1989), spatial autocorrelation analyses performed with the LCOR2 software (GOTTWALD et al, 1992b) showed aggregated disease patterns with a lack of directional spread of *Xac*, which indicated that the spread of *Xac* associated with splash dispersal of inoculum by irrigation was less than one meter from the source.

These results indicated the need to improve citrus nursery cultivation practices in Reunion nurseries to minimize CBCD transfer to new groves. Improvements to the present production scheme are as follows:
– mother plants for clean budwood should be grown in an insect-proof greenhouse;
– budded plants grown in plastic bags should be grown in tunnels and watered by drip irrigation, to avoid free water on the leaves, which promotes infection;
– budwood stock should be screened for asymptomatic *Xac* by IC-N-PCR.

durability of a clean planting stock strategy

Prior to deploying this strategy in commercial nurseries, we evaluated its durability in experimental groves planted with grapefruit or lime and produced under conditions specified above. All plants produced under such conditions remained free of CBCD lesions while in the nursery. Furthermore, all IC-N-PCR assays to detect asymptomatic *Xac* were negative. Therefore, it is likely that experimental plots were established free of *Xac*.

The durability of the improved production strategy was tested in two groves planted with the highly susceptible Henderson grapefruit (*Citrus paradisi*), in Saint-Pierre in April 1993, and in Saint-Benoît in March 1994.

the Saint-Pierre plot

The Saint-Pierre plot consisted of 117 trees bordered by windbreaks (*Casuarina equisetifolia*, *Leucaena leucocephala*, *Acacia auriculiformis*), and was located at the Cirad Bassin-Martin experimental research station (altitude 350 m, average annual rainfall approximately 1 000 mm). The experimental plot was surrounded by two CBCD-infected plots: clementine mandarins *(C reticulata)* located South of the experimental plot, and makrut lime *(C hystrix)* located Northwest of the experimental plot), and a CBCD-free plot: mandarins cv Beauty *(C reticulata)* located Southwest of the experimental plot. Meteorological events which could have contributed to the spread of *Xac* are presented in table I. All trees in the experimental grapefruit plot remained free of CBCD lesions until the end of February 1994, despite the occurrence of climatic conditions which were sometimes favorable to disease development. This confirms that *Xac* was probably not introduced in the experimental plot when it was established, but at a later date. There were several minor meteorological events between March and September 1993, with wind-driven rains mainly oriented in the Southeast direction (table I). Because there was no infected citrus upwind in relatively close proximity, these events did not lead to infection of trees in the grapefruit plot.

Hurricane Daisy, which moved westward 400 km North of the island, caused winds and rains over the experimental site on January 11, 1994 (table I). Again, wind-driven rains were mainly oriented to the Southeast. As a result, disease incidence in the neighboring infected plots strongly increased, but no CBCD lesions were recorded on grapefruit plants (disease assessment carried out on February 8, 1994).

Hurricane Hollanda, which travelled in a Southwest direction, came close to the Eastern coast of Reunion on February 10-11, 1994. Winds, first oriented to the Southeast, turned to the West on the morning of February 11. CBCD lesions were first recorded in the experimental plot at the end of February, and disease incidence increased rapidly during the following month. More than 300 *Xac* isolates were collected in the experimental and neighboring infected plots, frozen, and stored at –80 °C. Phage-typing using bacteriophages CP1, CP2, CP115, and CP122 (WAKIMOTO, 1967; WU et al, 1995) was performed on a subset of 170 isolates. Most isolates from grapefruit (91%) and from makrut lime (83%) were CP1–, CP2+, CP115+, CP122 +. The only other phage type recorded on these citrus species was CP1–, CP2–, CP115–, CP122+. All 30 isolates collected from Clementine mandarin had the following phage type: CP1+, CP2–, CP115–, CP122+. This information was consistent with the direction of wind-driven rains recorded during hurricane Hollanda, and indicated that the inoculum which contaminated the experimental grapefruit grove probably originated, at least in part, from the makrut lime grove.

the Saint-Benoît plot

The Saint-Benoît plot consisted of 135 trees and was located at the CFPPA experimental station (altitude 50 m, average annual rainfall approximately 4 000 mm). No other citrus were grown in this experimental station. The probability of CBCD infections associated with human transport via grove maintenance was therefore very small. Access to the plot was limited to only a few authorized persons. The experimental plot had no infected citrus in close proximity. A few infected citrus trees existed in private gardens located to the North-Northwest at least 500 m away from the plot. The majority of wind-driven rains recorded in this plot were oriented to the East-Southeast. Therefore, it was not expected that CBCD would develop unless the causal agent was disseminated by a hurricane with Northwest oriented wind driven rains or inadvertently transported by animals or insects.

The plot remained free of CBCD lesions until the beginning of October 1995, despite the occurrence of climatic conditions which were very favorable for disease development. This confirmed that planting materials used to establish the experimental plot were

Table I
Meteorological events at Saint-Pierre (Reunion) from plot establishment until citrus bacterial canker disease (CBCD) arrival.

Dates	*No of hours* [1]	*Wind speed* [2]	*Wind direction*	*Temperature* [3]	*Rainfall* [4]	*No of occurrences*
	1	4-5	NE-SE	<20	0.5-1	7
	2	4-5	SE	<20	0.5-2	2
	3	4-5	SE	<20	0.5-1	1
April to	8	4-5	SE	<20	0.5-1	1
September 1993	1	4-5	SE	20-26	0.5-1	3
	3	4-7	SE	<20	0.5-1	1
	2	4-6	SE	20-26	0.5-1	1
	1	5-6	E-SE	20-26	0.5-1	1
January 11, 1994	13	4-7	SE	20-26	12.5	–
February 10–11, 1994	32 [5]	4-11	SE, then W	20-26	155	–
End February 1994	92% of trees with CBCD lesions					

[1] Number of daily hours running in which wind-driven rains were recorded.
[2] Mean values within 1 h expressed in m/s.
[3] Mean values within 1 h expressed in °C.
[4] Expressed in mm.
[5] Number of hours running of wind-driven rains within 2 days.

probably not infected or contaminated with *Xac*. In October 1995, a few leaf lesions, all associated with citrus leaf miner galleries, were observed in two neighboring trees located in the middle of the border row on the Northwest side of the plot. Most wind-driven rains during the two months preceding infection were oriented to the Southeast. No wind-driven rains during that period were oriented to the North or Northwest. Therefore, dissemination of *Xac* from diseased trees located in backyard gardens was likely (table II). Infected trees were removed and destroyed by burning. Disease assessments were made on a more frequent basis during the months following the arrival of CBCD in the experimental plot. No further infections were recorded prior to the beginning of January 1997. CBCD was recorded on 17 trees, mostly located in the Northwestern portion of the grove. The age of these lesions was consistent with the occurrence of hurricane Daniela on December 7–8, 1996 (table II). However, the position of infected trees in the plot was not consistent with the direction of wind-driven rains generated by the hurricane. This indicated that *Xac* may not have been disseminated by wind-driven rains occurring during hurricane Daniela, but, rather, was present on these trees before that period (asymptomatically or as very few twig lesions). The climatic conditions during hurricane Daniela may have only promoted infection of *Xac*. The relationship between strains of the October 1995 and January 1997 outbreaks is presently under investigation by DNA fingerprinting and phage-typing.

conclusions

When analyzing the situation of CBCD in Reunion, VERNIÈRE (1992) stated that infected nursery plants constituted the main inoculum source for subsequent grove infections. Sensitive and specific molecular diagnostic tools based on IC-N-PCR are now available and are used, in association with improved nur-

-Table II
Meteorological events at Saint-Benoît (Reunion) for months preceding citrus bacterial canker disease (CBCD) arrival.

Dates	*No of hours* [1]	*Wind speed* [2]	*Wind direction*	*Temperature* [3]	*Rainfall* [4]	*No of occurrences*
	0.5	4-5	SE	<20	0.5-1.5	9
	0.5	6	SE	<20	0.5-2	9
	0.5	7	SE	<20	1.5	1
	0.5	8	SE	<20	1	1
	0.5	4-5	SE	20-24	0.5-1	8
	0.5	6	SE	20-24	0.5	2
July to	0.5	8	SE	20-24	0.5-1	2
September 1995	0.5	10	SE	20-24	0.5	1
	1	5-6	SE	<20	0.5-2	4
	1	6-7	SE	20-24	8	1
	1	7-8	SE	20-24	0.5	1
	5.5	5-10	SE	20-24	15	1
	0.5	4-5	SW	<20	0.5	2
	0.5	5	SW	<20	4.5	1
October 1995	2 infected trees (NW side of the grove)					
December 7-8, 1994	16 [5]	5-14	SE	20-24	135	
January 1995	17 trees with CBCD lesions					

[1] Number of daily hours running in which wind-driven rains were recorded.
[2] Mean values within 30 min expressed in m/s.
[3] Mean values within 30 min expressed in °C.
[4] Expressed in mm.
[5] Number of hours running of wind-driven rains within 2 days.

sery practices, for a better control of CBCD in citrus nurseries in Reunion. These detection techniques allow the production of CBCD lesion-free citrus plants in nurseries, which was not previously possible.

Commercial citrus production in Reunion has previously been restricted to mandarin cultivars owing to the presence of CBCD and other diseases which adversely affect other citrus types. There is a need to diversify commercial citrus production in Reunion. A common question raised by some citrus growers concerns the feasibility of planting acid citrus types (ie, combava, lime, grapefruit), which are generally very susceptible to CBCD. Preliminary experiments, conducted to evaluate the durability of a clean planting stock strategy, do not allow us to recommend such a choice. Our data indicate that the regular occurrence of hurricanes over Reunion and the recent introduction of citrus leaf miner in Reunion make it extremely difficult to continuously control CBCD on susceptible cultivars. The improvement of the citrus nursery production scheme will therefore essentially benefit citrus cultivars of low or moderate susceptibility to CBCD.

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Hacia un mejoramiento del control de la cancrosis de los cítricos en la isla de La Reunión.

RESUMEN

HISTORIAL DE LA ENFERMEDAD EN LA ISLA DE LA REUNIÓN. Las plantaciones industriales de cítricos en La Reunión, creadas con plantas procedentes de países sin cancrosis de los cítricos (CC), fecham de los años 60. Sin embargo, pudiera ser que la enfermedad hubiese estado presente en aquel entonces en la ista de La Reunión en algunos árboles de jardines criollos. El control de la CC en viveros podría permitir el mejoramiento de las condiciones sanitarias de las nuevas plantaciones. **DESARROLLO DE INSTRUMENTOS DE DIAGNÓSTICO.** *Xanthomonas axonopodis* pv *citri (Xac)*, diseminado por los jóvenes cítricos contaminados en viveros, es objeto de una serie de medidas internacionales de cuarentena, se ha desarrollado una técnica de detección específica y sensible a esta bacteria. Permite detectar el agente patógeno a partir de, aproximadamente, 10^2 cellulas/g de tejido foliar. **CONDICIONES CLIMÁTICAS DE LA ISLA Y DISEMINACIÓN DE *XAC* EN HUERTOS Y VIVEROS.** Las temperaturas y el nivel de precipitaciones son propicias, durante todo el año y en casi toda la isla, para la infección de las plantas por *Xac*. **ANÁLISIS ESPACIO-TEMPORALES DE LA PRESENCIA DE LA CC EN VIVEROS SIMULADOS.** Los análisis espacio-temporales de la evolución de la enfermedad evidenciaron una distribución agregativa del inoculó. Esta evolución esta más acentuadaen parcelas irrigadas por aspersión y asociada a las lluvias en las parcelas con irrigación localizada. Se ha elaborado, para minimizar la transmisión de la enfermedad en nuevas plantaciónes localizada. Se ha elaborado, para minimizar la transmisión de la enfermedad en nuevas plantaciones, un plan mejorado de producción local de plantones. **PERENNIDAD DE LA ESTRATEGIA DE PLANTACIÓN DE MATERIAL SANO.** Unos pomelos obtenidos con este plan se plantaron en diversas condiciones experimentales. La plantación de material sano se acompañó con otras medidas de lucha integrada. **CONCLUSIÓN.** El plan elaborado sólo es valido para la plantación de cítricos poco o medianamente sensibles a la CC.

PALABRAS CLAVES

Réunion, *Citrus*, epidemiologia, diagnóstico, *Xanthomonas axonopodis* pv *citri*.