LESSONS FROM A CASE OF SUCCESSFUL ERADICATION OF CITRUS CANKER IN A CITRUS-PRODUCING FARM IN SÃO PAULO STATE, BRAZIL

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SUMMARY

Asiatic citrus canker (ACC), caused by the bacterium *Xanthomonas citri* subsp. *citri*, is one of the most serious diseases affecting citrus worldwide. In areas under quarantine/eradication, such as São Paulo state (SPS), Brazil, the impact of ACC is related to the production costs for inspections and tree removal. Several protocols for eradication were adopted in SPS since the first detection of ACC in 1957. Among these, the one carried out from 1999 to 2009 was the most effective for maintaining the disease at a very low level throughout the state. That protocol mandated periodic inspections and removal of symptomatic and asymptomatic trees based on disease incidence in the block. After 2009 the eradication protocols were less stringent and ACC incidence continued to increase. This study aimed at identifying the key factors for the successful eradication of ACC in a farm located in SPS. Data on the outbreaks from 2006 to 2010 and the related control measures adopted were analysed. The farm remained under quarantine from July 2006 to March 2010. Twelve disease foci were detected from July 2006 to January 2008 and incidence of symptomatic tress ranged from 0.05% to 0.43%. The successful eradication of ACC in this farm is attributed to the relatively low number of diseased trees in all foci detected in the course of frequent and well-executed inspections. The results of this case study may be used to guide containment protocols in other citrus production areas of Brazil or countries where ACC is either absent or has a low incidence.

Keywords: *Xanthomonas citri* subsp. *citri*, legislation, inspection, plant disease foci, plant disease control.

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INTRODUCTION

Asiatic citrus canker (ACC), caused by *Xanthomonas citri* subsp. *citri* (*Xcc*), is a quarantine disease in many countries, where it is either absent or present but not widespread. In these areas, the control of ACC is ruled by official, compulsory measures of exclusion and eradication of the pathogen and marketing of citrus trees and fruits is regulated to prevent the spread of the disease to other citrus-producing areas. The quarantine status is due to the absence of *Xcc* in many citrus-producing regions of the world. Likewise, ACC is not present throughout Brazil (Behlau and Belasque, 2014). Thus, the disease has been under quarantine since it was first detected in the country in 1957 in São Paulo State (SPS) (Bitancourt, 1957). Immediately after ACC was confirmed in SPS, eradication efforts were undertaken to reduce the dissemination of the disease (Amaral, 1957). Plant eradication is a measure of control adopted within a geographical area (region, state, country, etc.) against an exotic pathogen that represents a threat to a crop when the disease incidence is low and no other more effective and less aggressive strategy is available. Often, not only the diseased plants are removed, but also the surrounding plants that are exposed to the inoculum (Kimati and Bergamin, 1995). However, not all plant diseases can successfully be controlled by eradication. Eradication of ACC is only feasible due to a set of epidemiological characteristics inherent to this pathosystem: (i) host range is restricted to the family Rutaceae, (ii) *Xcc* is not vectored by insects, (iii) symptoms develop quickly after infection, which allows for prompt detection and removal of diseased trees, (iv) *Xcc* cannot survive long outside of the host in the soil, weeds or organic matter, and (v) the pathogen is disseminated predominantly over short distances (Gottwald *et al.*, 2001; Kimati and Bergamin, 1995). Because citrus canker was never completely eliminated from SPS groves but was kept under very low incidence since its first detection, a suppression rather than an eradication of the disease was achieved statewide (Behlau and Belasque, 2014). The word eradication is used herein to refer to the control strategy adopted officially in SPS.

Several protocols for eradication have been attempted since ACC was first detected in SPS (Santos, 1991). Nonetheless, the protocol practiced from 1999 to 2009 is considered the most successful program conducted in SPS and the citrus production area of Minas Gerais state called Triângulo Mineiro (Belasque *et al*., 2009, 2010). During that time, the protocol quickly reduced ACC incidence in citrus blocks after an alarming increase in the late 1990's due to the introduction in 1996 of the citrus leafminer (CLM) (*Phyllocnistis citrella*), and maintained the incidence of new outbreaks at very low level (≤0.20% of affected blocks) for a decade (Belasque *et al*., 2010).

From 1995 to 1999, SPS legislation mandated the removal of symptomatic trees and those within a 30 m radius from a symptomatic tree (Anonymous, 1995). However, this protocol became less effective in the late 1990's, when the number of symptomatic trees in the state increased and the spatial pattern of diseased trees became less aggregated due to the interaction with CLM (Gimenes-Fernandes *et al*., 2000; Gottwald *et al*., 2007). Removal of trees within the 30 m radius was not as effective for eliminating the disease or reducing its spread as it was before the arrival of CLM. This is mainly due to the fact that CLM wounds increase the likelihood of *Xcc* infection to trees located at a higher distance from the inoculum source, so that the distribution of diseased trees in the foci becomes less aggregated (Jesus Jr. *et al*., 2006; Gimenes-Fernandes *et al*., 2000). Wounds caused by the feeding of CLM larvae on young leaf and stem tissues remain susceptible to *Xcc* infection for longer periods than mechanical wounds (Chagas and Parra, 2000; Jesus Jr. *et al*., 2006; Christiano *et al*., 2007). Moreover, CLM wounds are more easily infected than wounds caused by thorns, wind and trimming when aerosols disperse *Xcc* during rainstorms. Compared to infection through natural openings, 100 to 1000-fold less bacteria are necessary for infection through CLM wounds (Gottwald *et al*., 2002). Thus, despite of not being *Xcc* vector, CLM exacerbates the incidence and severity of ACC (Belasque *et al*., 2005; Hall *et al*., 2010; Jesus Jr. *et al*., 2006; Christiano *et al*., 2007).

Analysis of disease distribution within many citrus blocks affected by CLM in SPS revealed that the frequency of satellite foci increased significantly with incidences higher than 0.5% of symptomatic trees (Gottwald *et al*., 2007). Based on this data and the unprecedented increase of ACC incidence in SPS from 1997, a new methodology for eradication was established in June 1999 (Belasque *et al*., 2010; Gottwald *et al*., 2007; Anonymous, 1999), according to which an entire block was removed when the incidence of symptomatic trees exceeded 0.5%. In blocks with 0.5% incidence or less, only the symptomatic trees and those within a 30 m radius were removed.

In order to quantify the incidence of ACC in SPS in 1999, a statewide survey was undertaken in 10% of the blocks in the citrus-growing areas of the state and Triângulo Mineiro. Inspections and eradications were conducted by Fundecitrus, a private foundation funded by SPS citrus growers and juice industry, which was empowered by an agreement with the State Secretary of Agriculture (SSA). Although the Triângulo Mineiro is located in Minas Gerais state, this region is part of the SPS citrus industry, thus disease surveys were carried out as in the latter state. For the surveys: (i) one out of every five trees (20%) in a row was inspected in detail; and (ii) the remaining four trees (80%) were inspected continuously. For detailed inspections, surveyors walked slowly around the tree and stopped every two steps in order to scan the canopy thoroughly. For the continuous surveys, the inspectors walked alongside four trees in the row looking at the canopy without stopping until reaching the fifth tree, which was inspected in detail. In 1999, four thousand inspectors worked in the SPS eradication campaign and approximately 2 million trees were removed, which were either symptomatic or located in proximity of symptomatic trees.

In the following years (2000 to 2009), inspections were based on annual surveys, which were conducted in 5% of the citrus blocks, randomly selected within each citrus production region. The surveys in the selected blocks were performed as in 1999 for monitoring ACC dissemination progress and identifying new foci (Massari and Belasque, 2006). If a symptomatic tree was found during the survey, 100% of the citrus trees in the farm, as well as in the neighboring farms, were inspected in detail in order to detect foci of diseased trees as effectively as possible and remove them accordingly. If incidence of symptomatic trees in a citrus block was ≤0.5%, infected trees and those within a 30 m radius were removed; if incidence were >0.5%, the entire block was eliminated. Subsequently, a series of detailed inspections were undertaken to monitor the affected blocks as follows: (i) two 30-day-interval inspections by two teams each; (ii) two 60-day-interval inspections by two teams each; and (iii) as many as necessary 90-day-interval inspections by one team each until fulfilling two years of quarantine after the last detection. After this requirement was met, the grower was allowed to replant the block with citrus. During these inspections if incidence of diseased trees in the blocks was >0.5%, the entire block was eliminated. But if incidence was ≤0.5%, only the symptomatic trees were eliminated and the inspection cycle was started again. Therefore, the 30 m radius rule for eradication was only applied at first detection of the disease in blocks with ≤0.5% incidence. Additionally, all nurseries, and urban areas and non-commercial citrus groves in the most affected areas throughout the state were regularly inspected for removal of inoculum sources (Massari and Belasque, 2006; Anonymous, 1999).

This protocol was used until June 2009, when the eradication program in SPS was modified by Resolution SAA 43, which revived the guidelines used from the mid to late 1990s, in which the symptomatic trees and only the trees within a 30 m radius from an infected tree were eliminated, irrespective of the incidence of the disease in the block (Behlau and Belasque, 2014; Belasque *et al*., 2010). As the new regulation was less stringent and a greater number of inspectors would be required to keep the ACC incidence at low levels, the change led to the termination of the agreement between the SSA and Fundecitrus in January 2010. Since then, large scale inspections ended and disease eradication became the grower's responsibility with oversight by the SSA. The impact on the incidence of new ACC outbreaks was immediate. In the years following the change in the protocol the incidence of blocks with ACC went from 0.14% in 2009, to 0.44% in 2010, 0.99% in 2011, and 1.39% in 2012 (Behlau and Belasque, 2014; Belasque *et al*., 2010). In October 2013 the protocol was modified once again by Resolution SSA 147 and became even less rigorous. This resolution mandates that: (i) the trees located up to 30 m from the focus no longer need to be removed but sprayed with copper, (ii) only symptomatic trees are removed when disease is found; (iii) groves are inspected for ACC every three months, and (iv) growers submit semiannual reports to SSA listing the inspections and information on disease detection (Behlau and Belasque, 2014).

The recent changes in the methodology of eradication of ACC in SPS will likely result in a continue disease increase statewide which will probably discourage growers from further eradication efforts. Thus, for providing relevant information to citrus growers that choose to continue ACC suppression, this document reports a successful case of ACC eradication in a farm located in southern SPS and identifies the factors that were important to this aim. The study illustrates the history of ACC outbreaks and the measures adopted in a citrus-growing farm from the first detection of ACC until complete eradication of the disease was achieved. The case presented here may be useful for other citrus production areas in Brazil and countries where no ACC occurs or its incidence is at a low level, to decide whether eradication is the most effective and economical approach for disease control.

MATERIALS AND METHODS

Farm information. The case study was conducted in a citrus-growing farm named Quatro Irmãs, located in the municipality of Águas de Santa Bárbara, South SPS (longitude: 22° 52' 51" S, latitude: 49° 14' 20" O, altitude de 634 m a.s.l.). The climate of the region is highland tropical, with well-defined winter and summer seasons and average annual temperatures ranging from 15 to 28°C. Heat peaks in the summer are moderated by afternoon storms. The winter is dry and the average annual precipitation is 1,350 mm (Setzer, 1966).

The citrus-growing area in the farm consisted of 54,147 trees divided into 27 blocks, which were planted in 2003, 2004, and 2006 at a spacing of 7.0 × 3.5 m. Citrus blocks comprised sweet oranges (*Citrus sinensis*) cvs. Folha Murcha, Natal, Charmutt, Valencia and Lima Verde, and Murcott tangor (*C. reticulata* x *C. sinensis*) grafted on different rootstocks (Table 1). Fruit production was intended for domestic fresh market. The farm as well as the region where it is located had no previous records of ACC detection, which made the area an ideal location for this study.

Table 1. Citrus blocks cultivated in the studied farm from 2004 to 2010.

Block ID[a]	No. trees	Cultivar		Year of planting
		Scion[b]	Rootstock	
1A	2200	Folha Murcha	Rangpur Lime	2003
1B	2132	Folha Murcha	Rangpur Lime	2003
2A	2217	Natal	Swingle Citrumelo	2003
2B	2054	Natal	Swingle Citrumelo	2003
3A	2678	Charmut	Rangpur Lime	2004
4A	2598	Charmut	Rangpur Lime	2004
5A	2107	Valência	Cleopatra Tangerine	2003
5B	2064	Valência	Cleopatra Tangerine	2003
6A	2186	Charmut	Swingle Citrumelo	2004
6B	2021	Charmut	Swingle Citrumelo	2004
7A	2519	Valencia	Cleopatra Tangerine	2004
8A	1750	Valencia	Cleopatra Tangerine	2004
8B	1821	Valencia	Cleopatra Tangerine	2004
9A	913	Folha Murcha	Rangpur Lime	2003
10A	2216	Murcott	Rangpur Lime	2004
11A	2370	Valencia	Swingle Citrumelo	2004
12A	2651	Lima Verde	Rangpur Lime	2004
13A	1349	Murcott	Rangpur Lime	2003
14A	912	Pera Rio	Sunki Tangerine	2004
15A	2140	Pera Rio	Sunki Tangerine	2004
16A	1864	Pera Rio	Sunki Tangerine	2004
16B	1986	Pera Rio	Sunki Tangerine	2004
17A	1696	Pera Rio	Sunki Tangerine	2004
17B	1610	Pera Rio	Sunki Tangerine	2004
18A	2340	Pera Rio	Sunki Tangerine	2004
18B	2335	Pera Rio	Sunki Tangerine	2004
19	1418	Pera Rio	Rangpur Lime	2006

[a] ID, identification;
[b] Except for cv. Murcott, which is a tangor (*Citrus reticulata* x *C. sinensis*), all others are sweet orange (*C. sinensis*) cultivars

Besides the inspections, other measures were practiced to prevent introduction of ACC or its further dissemination within the citrus blocks, including use of dedicated harvesting equipment and restricted access of vehicles and people to the farm. No copper sprays were carried out for ACC suppression.

Inspection and eradication of ACC. From 2004 to 2006, three to four of 27 blocks were randomly selected each year at Quatro Irmãs farm for inspection by the annual survey program conducted by Fundecitrus. In January 2006, ACC was detected in a farm adjacent to Quatro Irmãs during the annual survey program conducted by Fundecitrus. At that time, when the disease was discovered in a given property in SPS, the neighboring citrus farms were 100% inspected, irrespective of the annual survey. Thus, inspections were also performed throughout the Quatro Irmãs farm from July to August 2006, which led to the first discovery of the disease. From 2006 to 2010, inspections and eradication methods used followed the guidelines in which every disease positive block was visited systematically until there was no further detection of ACC

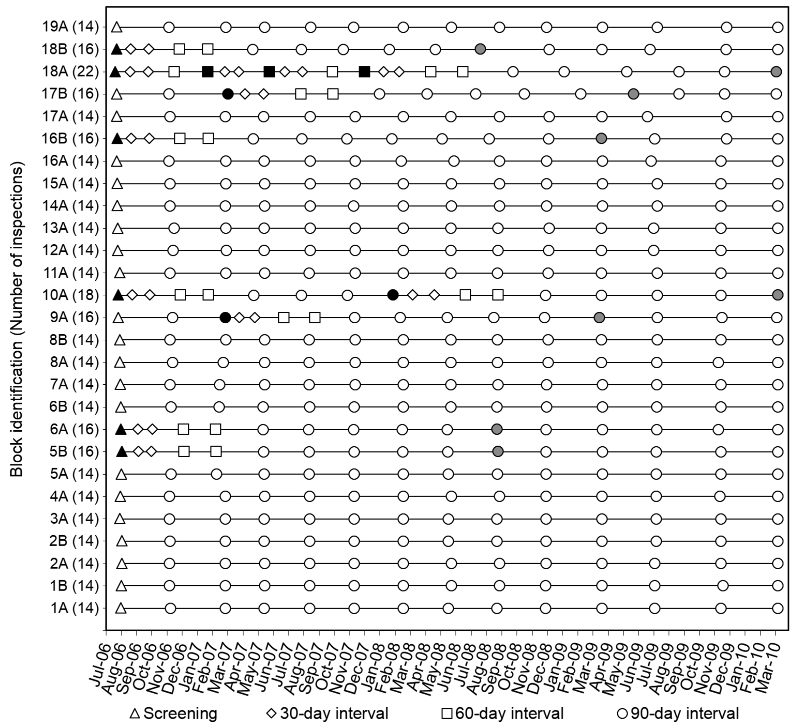

Fig. 1. History of inspections performed at Quatro Irmãs farm from the first detection to the eradication of the disease. (△) screening inspection conducted by three teams in each block in all trees to assess incidence of the disease in the block; (◇) 30-day interval inspection performed by two teams; (□) 60-day interval inspection performed by one team; (○) 90-day interval inspection performed by one team. Black and grey symbols indicate date of disease detection and end of quarantine, respectively. Total number of inspections in each block during the studied period is indicated within parentheses at axis *y* after block identification.

as ruled by the official protocol mentioned earlier (Fig. 1 and 2). In affected and non-affected blocks the inspection rates were 100 (performed in detail) and 700 (performed not in detail) trees/day/inspector, respectively. Inspections were carried out by a group of 20 trained scouts from Fundecitrus, who were also in charge of inspecting all citrus farms in that region of SPS.

Analysis of ACC foci. ACC outbreaks and eradication measures adopted in the farm were analyzed over time using information on the history of the disease in the region and neighboring farms, and several kinds of data were collected for each focus including date of detection, number of symptomatic trees, reoccurrence of the disease post-eradication, number of inspections, quarantine period, location and distance from other foci, and topography (Table 2). Based on the knowledge of ACC epidemiology and prediction of the origin of the inoculum, the most probable reasons for disease reoccurrence were hypothesized.

All ACC findings in the farm were numbered chronologically and designated either as primary focus (PF), for the first detection of the disease in the block, or as a secondary focus (SF), for the subsequent detections in blocks within the two-year quarantine period (Fig. 2). Therefore, all symptomatic trees detected in the same inspection cycle within a block belonged to the same PF or SF regardless the distance between them (Fig. 3).

RESULTS AND DISCUSSION

The first ACC symptomatic trees were detected in the farm on 21 July 2006, two years after the first blocks were planted (Table 2). During these first series of inspection, six foci designated PF1 to PF6 were found in blocks 18A, 18B, 16B, 10A, 6A and 5B, respectively. The number of trees with symptoms of the disease in these blocks totaled 21 and ranged from 1 to 10 in each block (Fig. 2 and 3A; Table 2). In none of the blocks did the incidence of diseased trees exceed 0.5%. Hence, only the symptomatic trees and those within 30 m around the symptomatic trees were removed. Overall, 1,326 trees were removed due to ACC detection in the first six PF (Table 2).

The inspection that led to the first detection of ACC in Quatro Irmãs farm was carreid out after the detection of the disease in a neighboring farm on 24 January 2006. ACC was introduced into the adjacent farm with infected citrus nursery trees. From January to May 2006, ACC outbreaks were observed in 13 nurseries of SPS, which were responsible for disease spreading to many areas where it had never been present before (Massari and Belasque, 2006), including the region of SPS where the farm was located. Over 16,000 trees were removed from the neighboring farm before the disease was last detected on 11 September 2006. Infected nursery trees are a very efficient means of ACC dissemination. After planting in the field, these trees serve as source of inoculum, which is released

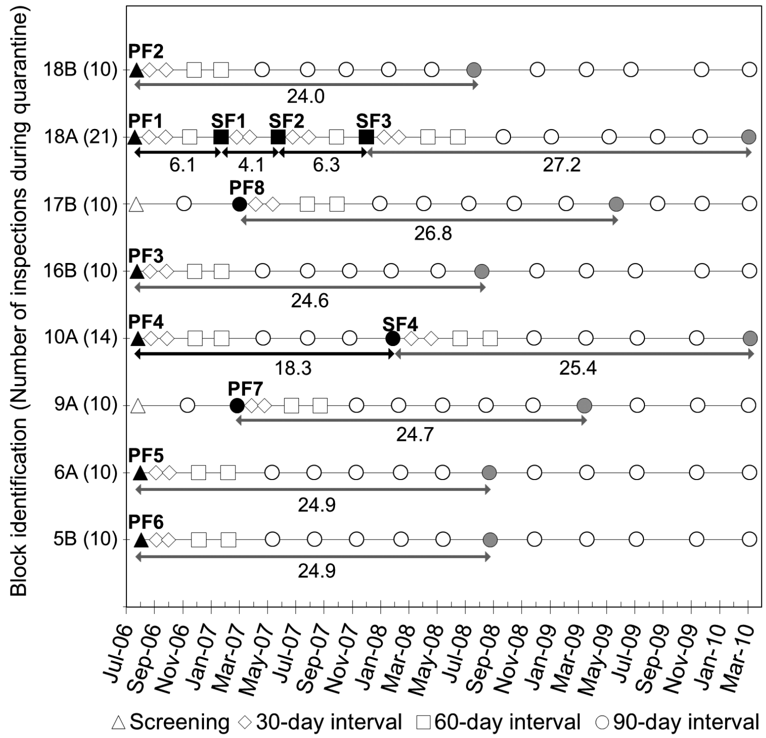

Fig. 2. History of primary foci (PF) and secondary foci (SF) of Asiatic citrus canker (ACC) at Quatro Irmãs farm from the first detection to the eradication of the disease. (△) screening inspection conducted by three teams in each block in all trees to assess incidence of the disease in the block; (◇) 30-day interval inspection performed by two teams; (□) 60-day interval inspection performed by one team; (○) 90-day interval inspection performed by one team. Black and grey filled symbols indicate date of disease detection and end of quarantine period in the block, respectively. Numbers below black and grey arrows indicate time in months until resurgence of ACC after eradication and mandatory time in months (≥2 years) with no detection of ACC after the last finding of the disease within the block, respectively. The sum of these periods is equivalent to the total quarantine period observed. Total number of inspections after the first ACC detection until the end of quarantine is indicated within parentheses at axis *y* after block identification.

from the preexisting lesions in the presence of water and transported to the surround trees by wind during rainstorms (Bock *et al*., 2010; Danós *et al*., 1984; Gottwald *et al*., 1992).

The number of symptomatic trees found in each of the six foci indicated that the disease probably arrived first in block 18A, where 10 trees (PF1) were detected during the first positive inspection (Fig. 2 and 3A; Table 2). This block was exposed to inoculum from the neighboring farm, which was located 600 m from the perimeter of the infected block and was set on a hillside facing the inoculum source (Fig. 3B). Usually, after introduction of *Xcc* from the outside source into a grove, ACC foci grows from one or a few initially infected trees and then disperses short distances to adjacent trees or blocks. Thus, the likelihood is that the greater the number of infected trees in a focus, the longer the disease has been present in that area. Moreover, unlike the other PF, symptoms of ACC in PF1 were observed not only on the leaves, but also on fruits and stems. This is another indication that the disease had been introduced at that site prior to other PFs. In areas under quarantine, lesions on fruits and especially on stems are less common and are usually followed by infection after inoculum build-up on the leaves. The remaining foci either originated from PF1, which was located 155, 418, 660, 1410, and 895 m from PF2, PF3, PF4, PF5 and PF6, respectively (Fig. 3A, Table 2), or from the inoculum in the neighboring farm where the disease was last detected a month after PF6 discovery.

Six months after the first detections and tree removal, a focus with three diseased trees, SF1, was found in block 18A (Fig. 3A, Table 2). SF1 originated either from PF1, located 60 m away, or from the neighboring farm. Because the inoculum in the neighboring area had been removed five months before the detection of SF1 and the fact that both PF1 and SF1 were located at a relatively short distance, the first possibility is more plausible. As for all SF that resulted an ACC incidence below 0.5% within the block, SF1 was eliminated by burning and removing only the symptomatic trees.

ACC-infected trees were detected again in the farm in two different blocks one month after the detection of SF1. The foci, named PF7 in block 9A and PF8 in block 17B, were detected in blocks with no disease history and contained one and two diseased trees, which resulted in the removal of 99 and 129 trees, respectively (Fig. 3A; Table 2). These were the last PF encountered in the farm. In PF7, the only diseased tree was located immediately adjacent

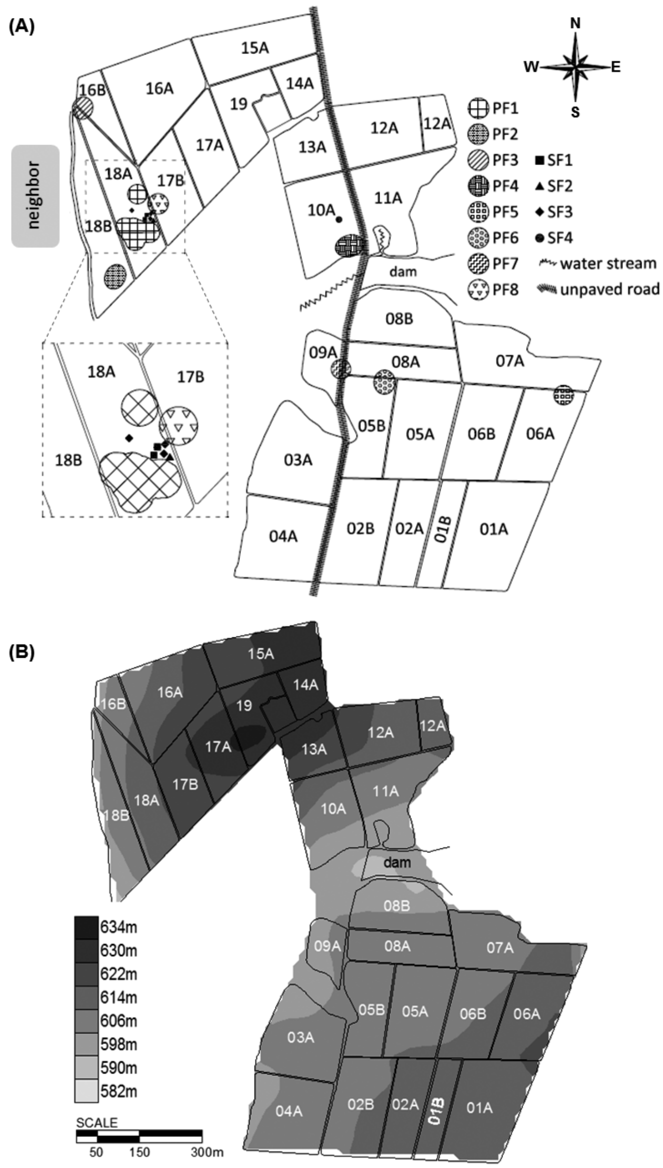

Fig. 3. Map of the Quatro Irmãs farm. (A) Location of primary foci (PF) and secondary foci (SF) of Asiatic citrus canker. (B) Topography map. Numbers within shapes indicate block identification. Block 18A is a close-up (dashed squares) to show more precisely the location of SF1, SF2, and SF3. "Neighbor" indicates the location where from the primary inoculum of citrus canker originated and spread to the studied farm.

to an unpaved road that splits the farm (Fig. 3A). As demonstrated elsewhere, the frequent movement of vehicles favors the occurrence of micro-injuries on plant surfaces by abrasion of plant tissue by dust particles, thereby increasing the opportunity for infection (Bock *et al.*, 2012). Possibly, PF7 originated from either PF6 (140 m) or PF1 (890 m). The first was the closest site to PF7 where ACC had been previously found, while the second contained more symptomatic trees (Fig. 3A; Table 2). Conversely, PF8 was located in block 17B, right next to 18A and only 100 m from FP1, which was very likely the source of inoculum (Fig. 3A).

Within approximately 11 months from PF8 detection, three other SFs were found in the farm. SF2 and SF3 were observed in block 18A in June and December 2007 and contained one and two symptomatic trees, respectively (Fig. 3A; Table 2). These trees were located at 44 to 56 m from PF1, the most probable inoculum source for these two SFs. SF4, detected in late January 2008, was the last disease finding on the farm (Fig. 3A; Table 2). This focus was located 88 m from PF4 in block 10A, where ACC had been detected one and a half years before. Because of this long interval and the fact that PF4 had only one diseased tree, it is more likely that SF4 originated from the SF of PF1, 627 m away, which were last detected a month before SF4.

Not coincidently, PF1 was located in the block most affected by ACC in the farm (18A). At first inspection, 10 symptomatic trees were found in PF1, which represents a 0.43% incidence within the block. If two additional infected trees had been found in this inspection, the block would have been completely removed because the incidence of symptomatic trees would have exceeded 0.5%. The relatively high disease incidence in this block substantially increased the time needed for achieving its eradication. Six other diseased trees were detected in block 18A in three different SFs (SF1 to SF3). It took 21 inspections and 43.7 months (foci detection + 2-year quarantine period with no disease detection in the block) to eradicate ACC in this block (Table 2). The number of inspections and time needed for eradication in 18A was double that in other affected blocks (except block 10A) where no more than three infected trees were identified at the first positive detection (Fig. 3A; Table 2).

The resurgence of ACC after several inspections and tree removals was very common in the eradication campaign in SPS (Belasque *et al.*, 2010). It has been demonstrated that more than a dozen consecutive inspections conducted by different groups of inspectors are necessary to detect all diseased trees remaining in a block after the 30 m radius eradication (Gimenes-Fernandes *et al.*, 2000). However, because inspections are costly and there is no guarantee that all diseased trees are identified, it is not feasible to conduct several concurrent inspections when a focus is found. A more sustainable strategy is to inspect the affected block several times throughout the year with one or few teams in a manner that the diseased trees that are not detected during a given inspection may be found in the subsequent survey cycles.

The reoccurrence of ACC or detection of a SF followed by removal of a PF was more prevalent after CLM became endemic in SPS. Before CLM, ACC dissemination was more aggregated and the 30 m eradication radius was relatively effective for removing all infected trees. In the presence of CLM, the reduced effectiveness of the 30 m radius and the increased likelihood that infected trees would escape eradication demanded more frequent inspections for disease elimination (Belasque *et al.*, 2010; Gottwald *et al.*, 2007; Jesus Jr. *et al.*, 2006; Christiano *et al.*, 2007).

Table 2. Information on history of Asiatic citrus canker (ACC) outbreaks in affected blocks.

Block ID[a]	PF[b]					SF[c]				End of quarantine	No. inspections during quarantine	Quarantine duration (months)
	Focus ID[a]	Detection date	No. diseased trees	% diseased trees	No. eradicated trees[d]	Focus ID[a]	Detection date	No. diseased trees	% diseased trees			
18A	PF1	21/Jul/06	10	0.43	580	SF1	24/Jan/07	3	0.17	12/Mar/10	21	43.7
						SF2	28/Jun/07	1	0.06			
						SF3	05/Dec/07	2	0.11			
18B	PF2	25/Jul/06	2	0.09	195	-	-	-	-	25/Jul/08	10	24.0
16B	PF3	26/Jul/06	2	0.10	145	-	-	-	-	11/Aug/08	10	24.6
10A	PF4	28/Jul/06	3	0.14	108	SF4	31/Jan/08	1	0.05	15/Mar/10	14	43.7
6A	PF5	02/Aug/06	1	0.05	137	-	-	-	-	27/Aug/08	10	24.9
5B	PF6	04/Aug/06	3	0.15	161	-	-	-	-	29/Aug/08	10	24.9
9A	PF7	28/Feb/07	1	0.11	99	-	-	-	-	21/Aug/09	10	24.7
17B	PF8	05/Mar/07	2	0.12	129	-	-	-	-	29/May/09	10	26.8

[a] ID, identification;
[b] PF, primary focus, first detection of ACC in a block;
[c] SF, secondary focus, subsequent detection of ACC in a previously affected block;
[d] For PF, the number of eradicated trees corresponds to the diseased trees and those within 30-m radius surrounding the focus. For SF, the number of eradicated trees is equal to the number of diseased trees. No block was entirely eliminated for exceeding 0.5% of trees with ACC after PF and SF detections.

Overall, the farm remained under quarantine for 3 years and 8 months (from July 2006 to March 2010) (Fig. 3A; Table 2). The relatively small number of diseased trees detected in all foci was a key factor for the successful eradication of ACC in the area. Within an 18-month period (July 2006 to January 2008), eight PF and four SF where detected in the farm with a total of 24 and 7 symptomatic trees, respectively (Table 2). The average number of diseased trees for each PF and SF was 3.0 and 1.8, respectively, and the ACC incidence did not exceed 0.5% in any affected block. Thus, although no block had to be entirely removed, the results observed for block 18A, confirm the importance of the 0.5% threshold for maintaining the ACC incidence of citrus blocks below 0.2% in SPS for a decade (Behlau and Belasque, 2014; Belasque *et al.*, 2010).

The low number of infected trees detected in the farm studied is a consequence of frequent, well-performed inspections and the 30 m eradication radius applied in all PF. Usually, the greater the number of diseased trees in a focus, the longer the distance of infected trees from the center of that focus. A low number of diseased trees in a PF increases the probability of eliminating all infected trees by applying the 30 m eradication radius (Gimenes-Fernandes *et al.*, 2000). Thus, in spite of the Resolution SSA 147, which since 2013 mandates the eradication of only ACC-infected trees, it is rational to keep removing asymptomatic trees, or even entire blocks, when more than a dozen of infected trees is detected. As presented here, all citrus trees in Quatro Irmãs farm were frequently inspected during almost four years. The average inspections per block and the total number of inspections in the farm were 14.9 (from 14 to 22) and 402, respectively, in the period covered by the eradication program in this farm. Blocks with ACC were inspected from 16 to 22 times (17 inspections per block in average). The frequent inspections of these blocks led to early detection of ACC and successful eradication by applying the 30-m tree removal radius for preventing further spread of *Xcc.*

Besides the exemplary inspections conducted after the disease arrived in the farm, two other actions significantly contributed to the elimination of the disease from Quatro Irmãs: (i) the neighbor farm was promptly inspected and trees removed, preventing further *Xcc* dissemination, and (ii) the inspections of Quatro Irmãs farm began immediately after ACC was found in the neighboring farm and all detected foci were quickly removed.

Successful eradication programs rely on extensive and timely actions by an official agency like Fundecitrus in SPS up to 2010. Although the reasons for the recent changes of the eradication protocol adopted in SPS are not the subject of the present study, it is reasonable to presume that the recent modifications will lead to a dramatic increase in ACC spread in SPS. Since January 2010, inspections for ACC and tree elimination in SPS became the grower´s responsibility. However, the efforts of thousands trained scouts cannot be matched by grower self-inspections. Even in situations where citrus growers are able to afford inspections and eradicate foci the proximity to urban areas and adjacent neighbors with ACC will reduce the efficacy of disease suppression. Furthermore, according to the current legislation in effect since November 2013, growers should conduct one widescreen inspection every three months and, for the first time in the history of SPS eradication program, the elimination of symptomless trees surrounding the diseased ones is no longer mandatory. Instead, trees located up to 30 m from the infected ones are to be sprayed with copper bactericides. This protocol could be effective only if conducted with numerous high quality inspections (≥ 12/year) and elimination of external sources of inoculum. Because these conditions are very unlikely to be met, the present ACC epidemic is expected to continue to spread in SPS.

SPS growers in ACC-free regions should be vigilant and avoid the introduction of *Xcc.* Nonetheless, if the disease is introduced into a new citrus-growing area and the incidence is low, it is reasonable to establish an inspection and tree removal program to prevent further ACC spread. We hope the experience in SPS and the results presented here can be used to shape future strategies for eradicating or suppressing ACC elsewhere in Brazil or in other countries.

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