Molecular Plant Pathology

MOLECULAR PLANT PATHOLOGY (2018) 19(6), 1302–1318 DOI: 10.1111/mpp.12638

Pathogen profile

Recent advances in the understanding of *Xanthomonas citri* ssp. *citri* pathogenesis and citrus canker disease management

CHRISTOPHER M. FERENCE[1,2], ALBERTO M. GOCHEZ[3], FRANKLIN BEHLAU[4], NIAN WANG[5], JAMES H. GRAHAM[6] AND JEFFREY B. JONES[2,*]

[1]*United States Department of Agriculture, Agricultural Research Service, US Horticultural Research Laboratory, Fort Pierce, FL 34945, USA*
[2]*Department of Plant Pathology, University of Florida, Gainesville, FL 32611, USA*
[3]*Citrus Pathology, EEA INTA Bella Vista, Bella Vista, Corrientes 3432, Argentina*
[4]*Department of Research & Development, Fundo de Defesa da Citricultura (Fundecitrus), Araraquara, São Paulo 14.807-040, Brazil*
[5]*Department of Microbiology and Cell Science, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, USA*
[6]*Department of Soil and Water Science, Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850, USA*

SUMMARY

Taxonomic status: Bacteria; Phylum Proteobacteria; Class Gammaproteobacteria; Order Xanthomonadales; Family Xanthomonadaceae; Genus *Xanthomonas*; Species *Xanthomonas citri* ssp. *citri* (*Xcc*).

Host range: Compatible hosts vary in their susceptibility to citrus canker (CC), with grapefruit, lime and lemon being the most susceptible, sweet orange being moderately susceptible, and kumquat and calamondin being amongst the least susceptible.

Microbiological properties: *Xcc* is a rod-shaped (1.5–2.0 × 0.5–0.75 μm), Gram-negative, aerobic bacterium with a single polar flagellum. The bacterium forms yellow colonies on culture media as a result of the production of xanthomonadin.

Distribution: Present in South America, the British Virgin Islands, Africa, the Middle East, India, Asia and the South Pacific islands. Localized incidence in the USA, Argentina, Brazil, Bolivia, Uruguay, Senegal, Mali, Burkina Faso, Tanzania, Iran, Saudi Arabia, Yemen and Bangladesh. Widespread throughout Paraguay, Comoros, China, Japan, Malaysia and Vietnam. Eradicated from South Africa, Australia and New Zealand. Absent from Europe.

Keywords: Asiatic citrus canker, *Xanthomonas axonopodis* pv. *citri*.

INTRODUCTION

Xanthomonas citri ssp. *citri* is a member of the family Xanthomonadaceae, one of the largest and most important groups of bacterial phytopathogens, and has been used as a model organism for the study of pathogenesis and phylogeny. Investigations of the pathogenome of *X. citri* ssp. *citri* have provided insights into bacterial phytopathogen host specificities, plant host recognition of pathogens, the means by which a plant host's cellular machinery is altered by the pathogen to facilitate infection, and pathogen propagation and dissemination. Analysis of the chromosomal and plasmid DNA of *X. citri* ssp. *citri* has helped to establish the evolutionary relationship between xanthomonad pathovars.

Xanthomonas citri ssp. *citri* is the causative agent of citrus canker (CC) disease and, as a pathogen of a globally important fruit crop, *Citrus*, has been the subject of extensive study with respect to epidemiology and disease management. Practices to exclude or quarantine *X. citri* ssp. *citri* continue to be refined wherever citrus is cultivated. Methodologies and products to manage and eradicate CC continue to be developed around the world.

In this review, we present the most recent findings and developments in the study of *X. citri* ssp. *citri* and CC, including in the areas of taxonomy, detection, the pathogenome, host–pathogen interaction, epidemiology, biofilm formation and management.

TAXONOMY OF CC XANTHOMONADS

CC, also referred to as Asiatic CC, was first observed in the USA during an outbreak in several southeastern states in the early 1900s (Stevens, 1914). Hasse received samples from Florida, Texas and Mississippi in 1914 and successfully isolated the bacterium (Hasse, 1915). Following characterization and pathogenicity experiments, Hasse named the bacterium *Pseudomonas citri* (Hasse, 1915). The bacterium has since been placed in various genera, including *Bacterium*, *Phytomonas* and, finally, *Xanthomonas* in 1939 as *Xanthomonas citri* (Doidge, 1916; Dowson, 1939; Jehle, 1916; Society of American Bacteriologists *et al.*, 1923). Strains within this species have been referred to as A strains to indicate that they are associated with Asiatic CC. In the 1970s, two additional CC-producing xanthomonads were identified and initially designated as group C strains, which cause canker lesions only in Key lime (*Citrus aurantifolia*), and group B strains, which

* *Correspondence*: Email: jbjones@ufl.edu

© 2017 BSPP AND JOHN WILEY & SONS LTD

have a wider host range (Namekata and Oliveira, 1972; Rosetti, 1977).

The bacterium remained in *X. citri* until 1978, when it was placed in *X. campestris* pv. *citri* by Dye in order to preserve *citri* at the infrasubspecific level (Young *et al.*, 1978). The bacterium was then transferred to *X. citri* by Gabriel *et al.* (1989), and the B and C strains were placed in *X. campestris* pv. *aurantifolii*. Young *et al.* argued that further work was needed relating to the placement of the CC strains in *X. citri* and therefore recommended that the A, B and C strains continue to be designated as *X. campestris* pv. *citri* (Young *et al.*, 1991). Using DNA–DNA hybridization (DDH) based on renaturation rates with a diverse array of *Xanthomonas* strains, Vauterin *et al.* (1995) transferred the A strains to *X. axonopodis* pv. *citri* and the B and C strains to *X. axonopodis* pv. *aurantifolii*.

Schaad *et al.* (2005) used the S1 nuclease technique for DNA–DNA relatedness to place the *X. axonopodis* pv. *citri* strains in *X. smithii* ssp. *smithii* and the *X. axonopodis* pv. *aurantifolii* strains in *X. fuscans* ssp. *aurantifolii*. The placement of strains in *X. smithii* was later deemed illegitimate because of the preceding legitimate proposal by Gabriel *et al.* (1989). Schaad *et al.* (2006) then emended their original recommendation, placing these strains in *X. citri*. In 2007, *X. citri* ssp. *citri* was formally validated (Euzeby, 2007).

Recently, Constantin *et al.* (2016) have proposed significant changes to xanthomonad taxonomy, including within *X. citri*. Using a polyphasic approach which included multilocus sequence analysis (MLSA), DDH calculation of whole-genome average nucleotide identity values and phenotypic analyses, they recommended the addition of a number of pathovars within *X. axonopodis*, as well as placement of members of *X. fuscans*, into *X. citri*. As such, *X. fuscans* ssp. *aurantifolii* has been recommended to be transferred to *X. citri* as *X. citri* pv. *aurantifolii* (Constantin *et al.*, 2016). The authors have submitted their recommendations to the International Journal of Systematic and Evolutionary Microbiology for these changes, and they have been approved (M.-A. Jaques, personal communication). For now, *X. citri* ssp. *citri* (*Xcc*) and *X. fuscans* ssp. *aurantifolii* (*Xfa*) are the most recent prokaryotic names with standing in nomenclature for those bacteria that cause CC.

DETECTION AND IDENTIFICATION OF XANTHOMONADS CAUSING CITRUS BACTERIAL CANKER

There are several techniques used for the diagnosis of CC. However, in most cases, when no official confirmation is needed, the disease can be identified by the recognition of typical symptoms (Behlau and Belasque, 2014; Gottwald *et al.*, 2002; Schubert *et al.*, 2001). Isolation of *Xcc* from lesions on solid medium, followed by the observation of xanthomonad-like colonies, i.e. yellow, convex, circular, semi-translucent and with regular margin, may also be used to confirm the causal agent (Schaad *et al.*, 2001). Pathogenicity tests on susceptible citrus species may be performed by the infiltration of a bacterial suspension adjusted to 10^8 colony-forming units (CFU)/mL into the leaf mesophyll, followed by the observation of water soaking and raised margins in the infiltrated portion of the leaf 2–4 days after inoculation (Behlau and Belasque, 2014; Schubert *et al.*, 2001).

When symptoms are atypical or an official confirmation is necessary for quarantine purposes, DNA-based assays and serological tests are commonly used methods for CC diagnosis. Although molecular methods are able to detect the presence of *Xcc* in infected plant tissue, even before the appearance of canker lesions, serology-based tests are usually sufficient for *Xcc* detection from symptomatic tissue. Several sets of primers have been designed for polymerase chain reaction (PCR) detection of *Xcc* based on rDNA sequences, plasmid-borne genes and pathogenicity regulatory factors (Coletta-Filho *et al.*, 2006; Cubero and Graham, 2002, 2004, 2005; Hartung, 1992; Mavrodieva *et al.*, 2004; Sun *et al.*, 2004). More recently, the development of real-time PCR and loop-mediated isothermal amplification protocols has increased the accuracy of diagnostic tests for the detection of *Xcc* (Cubero and Graham, 2005; Golmohammadi *et al.*, 2007; Mavrodieva *et al.*, 2004; Park *et al.*, 2006; Rigano *et al.*, 2010). Rep-PCR with BOX and ERIC primers has also been used to differentiate strains within the same *Xcc* pathotype (Cubero and Graham, 2002; Jaciani *et al.*, 2012; Louws *et al.*, 1999). The serology-based test, also known as enzyme-linked immunosorbent assay (ELISA), is based on the ability of an antibody to recognize and bind to a specific antigen, in this case a substance associated with *Xcc*. ELISA has been demonstrated to be useful for the rapid diagnosis of CC (Alvarez *et al.*, 1991; Bouzar *et al.*, 1994; Civerolo and Fan, 1982; Gottwald *et al.*, 1991; Sun *et al.*, 2004). This test is usually performed in the laboratory, but is also available as a strip-based kit easy to use in the field, where disease is suspected. These kits do not require special equipment or training to use and the results are obtained within a few minutes (Al-Saleh *et al.*, 2014).

Other older techniques for the detection of *Xcc* have been developed. These include physiological characterization, fatty acid profile analyses, protein profiling, hybridization, restriction fragment length polymorphism analysis and comparison of plasmid DNA patterns (Bouzar *et al.*, 1994; Egel *et al.*, 1991; Gottwald *et al.*, 1991; Hartung, 1992; Pruvost *et al.*, 1992; Sun *et al.*, 2004; Vauterin *et al.*, 1991a,b, 1996a,b; Verniere *et al.*, 1991, 1998).

XANTHOMONAD PATHOGENOME

A major landmark in the characterization of *Xcc-A* was the complete genome sequencing of strain *Xcc*-A306 in 2002 (da Silva *et al.*, 2002). The genome of this strain consists of a 5 175 554-

base pair (bp) chromosome encoding approximately 4500 genes, and two plasmids (da Silva *et al.*, 2002). The single circular chromosome has a 64.7% G + C content, and 4314 predicted open reading frames (ORFs) (Van Sluys *et al.*, 2002). The two plasmids, pXAC64 (64 920 bp) and pXAC33 (33 699 bp), have 61.4% and 61.9% G + C content, respectively (da Silva *et al.*, 2002; Van Sluys *et al.*, 2002). Approximately 7% of *Xcc* genes are involved in pathogenicity, virulence and ecological adaptation (Van Sluys *et al.*, 2002).

Some features discovered in the *Xcc-A* genome were the large number of cell wall-degrading enzymes (CWDEs), proteases, iron receptors, genes related to energy metabolism pathways, the type 2 secretion system (T2SS) and type 3 secretion system (T3SS), genes for flagella structural units and chemotactic protein genes, and the xanthomonadin and xanthan gum synthesis gene cluster (*gumB* to *gumM*), which are important in the epiphytic phase of the life cycle (Dunger *et al.*, 2007). There are genes for the production of biofilms, which facilitate the formation of bacterial aggregates in the apoplast, proliferation of the bacterium and subsequent symptom development (Rigano *et al.*, 2007).

The 23-kb *hrp* (for *hypersensitive reaction and pathogenicity*) region has six operons, designated as *hrpA* to *hrpF* (Rossier *et al.*, 2000). The *hrp* cluster is part of a pathogenicity island in the *Xcc* genome and encodes the T3SS. This cluster also possesses transposases and has a different G + C content from the rest of the genome, providing evidence for the acquisition of the *hrp* cluster via horizontal gene transfer (Hacker and Kaper, 2000). The left border of the core region (*hrpA* to *hrpE*) carries different species-dependent effector genes, which contribute to pathogenicity on different hosts. The *hrpB* operon, which encodes eight proteins, has been demonstrated to be very stable at the species level, and its sequence can be used for phylogenetic analysis and species determination in xanthomonads (Leite *et al.*, 1994; Obradovic *et al.*, 2004). The right border, called the HrpF peninsula, is the more variable subregion among xanthomonads in terms of overall structure and gene content (Tampakaki *et al.*, 2010). The *Xcc hrpG* and *hrpX* genes collectively regulate all 24 T3SS genes, 23 T3SS effector genes and 29 T2SS substrate genes, as well as XacPNP (Guo *et al.*, 2011). T2SS substrate products include proteases, lipases and CWDEs. The genes *hrpG* and *hrpX* are involved in the regulation of amino acid biosynthesis, oxidative phosphorylation, the pentose-phosphate pathway, phenolic catabolism and the transport of sugar, iron and potassium in response to exposure to the host environment (Guo *et al.*, 2011). An additional 124 and 90 unknown genes are regulated by *hrpG* and *hrpX*, respectively (Guo *et al.*, 2011). HrpG induces the expression of 11 proteins secreted by the T2SS, as well as being a regulator of the T3SS (Yamazaki *et al.*, 2008a). Together with HrpG, another T3SS regulator, HrpXct, is involved in the *in planta* multiplication of *Xcc* (Yamazaki *et al.*, 2008b).

After sequencing the *Xcc*-A306 genome, the São Paulo State Science Foundation (FAPESP) Genome Program sequenced the genomes of *Xfa-B* (strain B69) and *Xfa*-C (strain Xc70) (Moreira *et al.*, 2010). The characterization of the sequenced genomes revealed genes for T3SS effectors, type 4 secretion system (T4SS) proteins, biofilm formation, quorum sensing (QS), sugar acquisition, flagellum construction and lipopolysaccharide (LPS) synthesis. The genomes of the *Xfa-B* and *Xfa-C* strains were compared with the *Xcc*-A306 genome as a reference. In general terms, the *Xfa-B* strain genome had more similarities with the *Xcc*-A306 genome (87%) than with the *Xfa-C* strain (84%). The *Xfa-B* strain genome contained more T3SS effectors than the *Xfa-C* strain, and some specific T3SS effectors were shared between *Xcc-A* and *Xfa-B*, but were not found in *Xfa-C*. A few T3SS effectors were found only in *Xfa-C*, showing a divergence in the pathogenic mechanisms developed for each strain. Compared with *Xcc*-A306, both *Xfa-B* and *Xfa-C* shared 46% of the sequence of the *Xcc* plasmid pXAC33, the *Xfa-B* strain shared 61% of the *Xcc* plasmid pXAC64 sequence, whereas the *Xfa-C* strain shared only 55% of pXAC64.

A comparative genomic analysis of strains *Xcc-A306* and *Xcc-Aw12879*, an A strain isolated in Florida and pathogenic on Key lime and alemow (Sun *et al.*, 2004), indicated that the *xopAG-avrGf1* gene in *Xcc-Aw12879* contributed to the host range specificity in *Xcc-A*^{w} (Jalan *et al.*, 2013b). Other effectors present in *Xcc-A*^{w}, such as XopAF (which is also found in *Xfa-B* and *Xfa-C*), contribute to the virulence of *Xcc-A*^{w} (Jalan *et al.*, 2013a). Although the complete genome of *Xcc-Aw12789* shows a close relationship with *Xcc-A306*, numerous inversions and translocations between both genomes were found. *Xcc-A*^{w} also possesses two plasmids: pXacw19, which does not have any similarity with *Xcc*-A306, and pXacw58, which carries two transcription activator-like effector (TALE) genes, denoted *pthAw1* and *pthAw2*.

In 2010, several *Xcc-A*, *Xcc-A** (produces canker lesions in Mexican lime but not grapefruit; Verniere *et al.*, 1998), *Xcc-A*^{w} and *X. citri* pv. *bilvae* (which causes CC-like symptoms in Key lime) strains were characterized by amplified fragment length polymorphism (AFLP) and MLSA based on four partial housekeeping gene sequences (*atpD*, *dnaK*, *efp* and *gyrB*). Based on the high genetic relatedness amongst the strains, the authors suggested new nomenclature for one of the strains of *X. citri* pv. *bilvae*, which is now considered as a junior synonym of *Xcc-A** strains. The authors also demonstrated a close relationship between *Xcc-A*^{w} and *Xcc-A** strains, which were proposed as a highly variable subgroup of *Xcc-A* strains (Ngoc *et al.*, 2010). Recently, a complete genome comparison (pan-genome analysis) of 25 sequenced strains of *Xcc-A*, *Xcc-A*^{w} and *Xcc-A** has revealed 85% similarity in the genome between these three groups (Jalan *et al.*, 2013a,b). Most of the variation between groups was

observed between *Xcc-A*^{w} and *Xcc-A** versus typical *Xcc-A* strains. The variation was associated principally with chromosome deletions, transposition events and plasmid insertions.

A molecular database generated by AFLP analysis of 55 previously characterized *Xcc-A* strains was used to elucidate which factors determined the differential host pathogenicity in *Xcc-A** strains (Escalon *et al.*, 2013; Ngoc *et al.*, 2009). In this work, 66 T3SS effectors were identified, where 28 were common to *Xcc-A*, *Xcc-A** and *Xcc-A*^{w}. Two of the effectors, XopAG and XopC1, appeared to be limited to *Xcc-A** and *Xcc-A*^{w} strains. Interestingly, the T3SS effector XopAG-*avrGf1* was found in all strains of *Xcc-A*^{w} and *Xcc-A** which were only pathogenic in key lime, whereas XopC1 was found in only four of the *Xcc-A** strains that showed limited host range. The effector XopC1 does not elicit a hypersensitive response (HR), nor does it affect the pathogenicity of the strains after its deletion. Different combinations of T3SS effectors present in the strains were used to create phylogenetic trees to explain the limited host range of *Xcc-A** strains, but, except for the presence of the gene *avrGf1*, were not able to strictly correlate the presence of a specific combination of effectors with the reduced host range. The *Xcc-A** strains appear to be the most highly diverse group, with greater diversity than has been observed in *Xcc-A* strains. It has been suggested that the presence of homologous T3SS effectors in *X. citri* pv. *bilvae* is the result of fluid horizontal genetic exchange between different *Xanthomonas* species (Escalon *et al.*, 2013).

Several *Xcc* strains (14 *Xcc-A*, three *Xcc-A** and four *Xcc-A*^{w}) collected from North America and Asia were sequenced, and comparative genomic and evolutionary analyses were conducted (Zhang *et al.*, 2015). The authors calculated the pan-genome of these 21 *Xcc* strains using the OrthoMCL method. The core genome comprised 3912 orthologous clusters, whereas the pan-genome contained 5147 orthologous clusters. Based on the genome analysis, *Xcc-A* strains formed a separate clade from the *Xcc-A*^{w} and *Xcc-A** strains, as revealed by the phylogenetic tree reconstructed on the basis of the core genome, the phyletic distribution of accessory orthologous clusters of the strains analysed, and the different origin of genomic islands of the two groups. This finding is consistent with a previous report based on multilocus variable number of tandem repeat analysis (Pruvost *et al.*, 2015). However, these results differ from other results, which suggest that the strains from each pathotype form monophyletic clades, with a short branch shared by the *Xcc-A*^{w} and *Xcc-A* pathotypes (Gordon *et al.*, 2015). Such differences in the investigations of *Xcc* phylogeny call for further studies to understand the evolution of *Xcc* strains (Gordon *et al.*, 2015; Zhang *et al.*, 2015). It is presumed that the acquisition of beneficial genes and the loss of detrimental genes has most likely allowed *Xcc-A* to infect a broader range of hosts relative to *Xcc-A** and *Xcc-A*^{w}. These studies imply that the *Xcc* population is clonal in structure. Many genes related to virulence, especially genes involved in the T3SS and effectors, are affected by positive selection, further highlighting the contribution of positive selection to the evolution of *Xcc* (Zhang *et al.*, 2015).

A detailed study was performed on 157 *Xcc* strains from Brazil, which were compared for their T3SS effector profiles using a qualitative PCR-Southern blot technique (Jaciani *et al.*, 2012). Low genetic variability was observed for strains isolated in the northern part of the country, but more diversity was present in the strains isolated in the southern part, where the disease was more prevalent (Jaciani *et al.*, 2012). In China, several *Xcc* strains from nine citrus-growing regions were characterized and compared for the variability of TALEs (Ye *et al.*, 2013). The result of the analysis of 105 strains with differential pathogenicity on a set of citrus hosts showed that the strains varied in the number of TALEs, ranging from three to five *pthA* genes (Ye *et al.*, 2013). A comparison of the strains through hybridization with a probe based on the *Xcc*-A3213 *pthA* gene allowed the separation of the strains into 14 genotypes, with more than 80% of the strains being placed in two major groups (Ye *et al.*, 2013). The lack of hybridization observed in some strains was correlated with a lower virulence of these specific strains and could be correlated with variation in the sequences of the *pthA* genes (Lin *et al.*, 2005, 2011; Ye *et al.*, 2013). A slight modification in the number of repeats of the *pthA4* gene produced changes in the pathogenicity of the bacterium, specifically in the induction of CC symptoms (Lin *et al.*, 2013).

The host plant genes, whose products recognize pathogen effectors, are known as *R* genes, and the pathogen pathogenicity (*pth*) genes, which encode these recognizable effectors, are also referred to as avirulence (*avr*) genes. The products of *R* genes, either directly or indirectly, interact with the products of *avr* genes (Mysore and Ryu, 2004). The interaction between the products of *R* and *avr* genes is termed gene-for-gene resistance. No *R* genes corresponding to *Xcc*, and therefore imparting resistance to *Xcc* to the host plant, have been identified in citrus or citrus related species to date (Brunings and Gabriel, 2003; Khalaf *et al.*, 2007).

HOST–PATHOGEN INTERACTION AND INFECTION PROCESS

Xcc inoculum is deposited on host tissue via rain splash and is capable of swimming short distances with its flagellum. It then enters the host directly through wounds or through stomatal openings (Graham *et al.*, 1992). Once within the apoplastic space, the bacterium adheres to a host cell wall surface with either an *hrp* or T4SS pilus (Brunings and Gabriel, 2003; He, 1998). Once populations of *Xcc* achieve sufficient density, bacteria shed their flagella, transitioning away from a planktonic lifestyle and aggregating into a biofilm composed of the polysaccharide xanthan and other components (Costerton *et al.*, 1995; Graham *et al.*, 2004).

Within days of infecting a compatible host, *Xcc* elicits the characteristic hypertrophy and hyperplasia symptoms of CC (Schubert *et al.*, 2001).

PthA is a member of the *avrBs3*/*pthA* gene family (Brunings and Gabriel, 2003). PthA is a homologous protein to AvrBs3, which possesses a nuclear localization signal (NLS) and is translocated into the host cell via the T3SS and targets the nucleus, where it activates the expression of the host genome's *upa20* following binding to that gene's promoter (Kay *et al.*, 2007). Upa20 is a regulator of cell size and *avrBs3*, like *pthA*, reprograms infected cells by mimicking host transcription factors by binding to the conserved promoter region of *upa20* (Kay *et al.*, 2007). The activation of *upa20* by *avrBs3* is believed to be one of the mechanisms by which *Xcc* induces hypertrophy in susceptible hosts (Kay *et al.*, 2007). The hypertrophic and hyperplastic growth of host tissues is accomplished through the injection into the host cell of PthA (Brunings and Gabriel, 2003; Duan *et al.*, 1999; Swarup *et al.*, 1991; Yang and Gabriel, 1995). The expression of this single gene of *Xcc*, *pthA*, in citrus cells is sufficient to cause the division, enlargement and death of host cells (Duan *et al.*, 1999). The specific *pthA* gene present in strain *Xcc306*, referred to as *PthA4*, facilitated by its NLS, is conveyed to the host cell nucleus by the host cell's own machinery, where it mimics the host's own transcription factors activating the susceptibility gene, *LOB1* (lateral organ boundaries 1) (Hu *et al.*, 2014; Yang and Gabriel, 1995). This specific TALE sequence (*pthA4*) is responsible for the elicitation of CC symptoms through the activation of specific genes in the host (Shantharaj *et al.*, 2013). Mutations of *pthA4* result in a loss of hypertrophy, but have shown variation in the effect on bacterial growth in host leaf tissue (Figueiredo *et al.*, 2011a; Hu *et al.*, 2014; Yan and Wang, 2012).

PthA4 up-regulates the host gene *CsLOB-1*, a member of the LOB transcription factor family, and *CsSWEET1*, a homologue of the SWEET sugar transporter and rice disease susceptibility gene family (Fig. 1) (Hu *et al.*, 2013). PthA4 interacts *in planta* with the C-terminal domain (CTD) of RNA polymerase II, inhibiting the activity of a CTD-associated cyclophilin (Cyp), and with the protein CsMAF1, which represses tRNA synthesis and cell growth through its interaction with the RNA polymerase III (Domingues *et al.*, 2012; Soprano *et al.*, 2013). The importance of PthaA induction of *LOB1* was further confirmed when genome editing of *CsLOB1* rendered citrus resistant to CC (Jia *et al.*, 2017).

PthA2 and PthA4 target auxin and gibberellin synthesis in citrus, and recruit RNA polymerase II to initiate their targeted transcription (Pereira *et al.*, 2014). Whether through their regulation of auxin or gibberellin, or some other mechanism, host plants also increase ethylene production in response to PthA2 and PthA4 (Pereira *et al.*, 2014). Transiently expressed PthA2 individually regulates genes involved in DNA metabolism, replication and integration, whereas PthA4 individually regulates microtubule-based processes and movement, and, together, they regulate sugar metabolism, lipid transport and cell wall organization (Pereira *et al.*, 2014).

In addition to *pthA*, *Xcc* possesses three additional *pthA* (*PthA4*) alleles, two of which weakly elicit cankers and one that is non-functional (Brunings and Gabriel, 2003; Swarup *et al.*, 1992). The *pthA* homologue, hssB3.0, was found to be involved in the reduced virulence of *Xcc* in *Citrus grandis* (pomelo), and hssB3.0 was subsequently discovered in all weakly aggressive strains of *Xcc* (Shiotani *et al.*, 2007). The *pthA* homologues, *pthB*, *pthC* and *pthA*^{W}, are found in the *Xaa-B*, *Xaa-C* and *Xcc-A*^{W} strains, respectively, and are required for those strains to induce canker symptoms (Brunings and Gabriel, 2003; Al-Saadi *et al.*, 2007). The gene *pthA* and its homologues, when introduced into non-hosts, are suspected to induce HR in an *R* gene-based gene-for-gene interaction, suggesting a role for *pthA* in host specificity (Swarup *et al.*, 1992).

Experiments involving *Xcc* with mutations of *pthA*, however, differentiated *pthA* from other *hrp* genes in that, although *Xcc* mutants lost their ability to elicit an HR on one non-host, bean (*Phaseolus vulgaris* L. cv 'California Light Red'), the mutant bacterium retained its ability to elicit an HR on another non-host, cotton (*Gossypium hirsutum* L.) (Swarup *et al.*, 1992). *PthB* is found in *Xaa-B* on a plasmid, pXcB, which also possesses ORFs that encode a T4SS, for conjugation and horizontal exchange of plasmid DNA (El Yacoubi *et al.*, 2007).

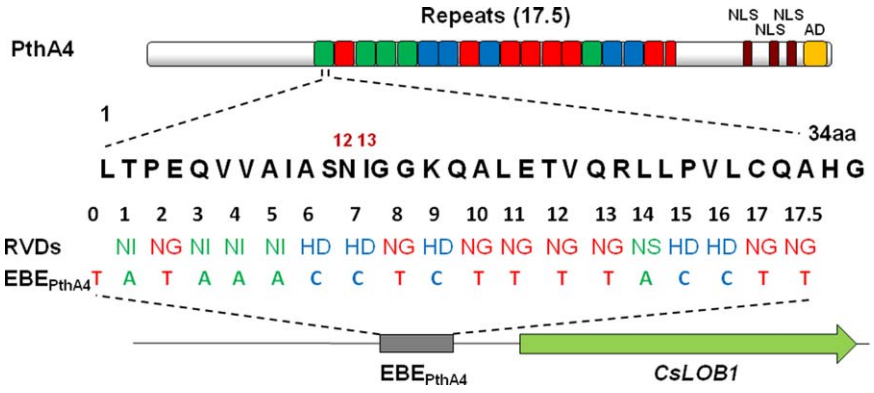

Fig. 1 The transcription activator-like effector (TALE), PthA4, contains 17.5 repeats in which the repeat-variable diresidues (RVDs) bind to the effector-binding element (EBE) upstream of the susceptibility gene, *CsLob1*, and turn on *CsLob1*. The arrangement of the RVDs dictates the target of TALE, which, in this case, is EBE$_{PthA4}$. aa, amino acid; NLS, nuclear localization signal.

As *Xcc*-infected host cells divide and enlarge, they come into closer contact with one another, reducing the free apoplastic space which is filled by hygroscopic xanthan produced by *Xcc* in response to QS by a small-molecule diffusible signal factor (DSF) (Brunings and Gabriel, 2003; Tang *et al.*, 1991). This causes the characteristic water-soaking symptoms around expanding lesions as capillary action brings in water which hydrates the xanthan (Popham *et al.*, 1993). PthA is also suspected of inducing limited host cell death to assist in the rupture of the epidermis, to provide additional nutrition for bacterial growth, or both. In addition to the increased production of xanthan, QS and DSF are believed to be involved in the up-regulation of additional *avr* genes after a certain population threshold, including those which increase the production of cellulases, proteases and pectinases (da Silva *et al.*, 2002). Although no *R* genes have been identified in citrus, the *avr* gene, *avrGf1*, a member of the XopAG effector family in *Xcc-A*^{w}, was shown to elicit an HR in grapefruit (Fig. 2B,C) (Rybak *et al.*, 2009). When the *hrpG* gene from *Xcc-A*^{w} was expressed in *X. perforans*, infection of grapefruit with the transconjugant resulted in an HR, whereas wild-type *X. perforans* normally elicits no response, indicating a yet unidentified HR-inducing factor (Rybak *et al.*, 2009). Transconjugants possessing *avrGf1*, but lacking a functional T3SS, failed to elicit HR in grapefruit (Rybak *et al.*, 2009). Likewise, *avrGf1* encodes a chloroplast localization signal (CLS) required for targeting the host cell's chloroplasts, and CLS knockout mutants failed to elicit an HR, indicating that both a functional T3SS and an intact CLS are required for *avrGf1* to act as an effector in host cells (Fig. 2A,D) (Figueiredo *et al.*, 2011b;

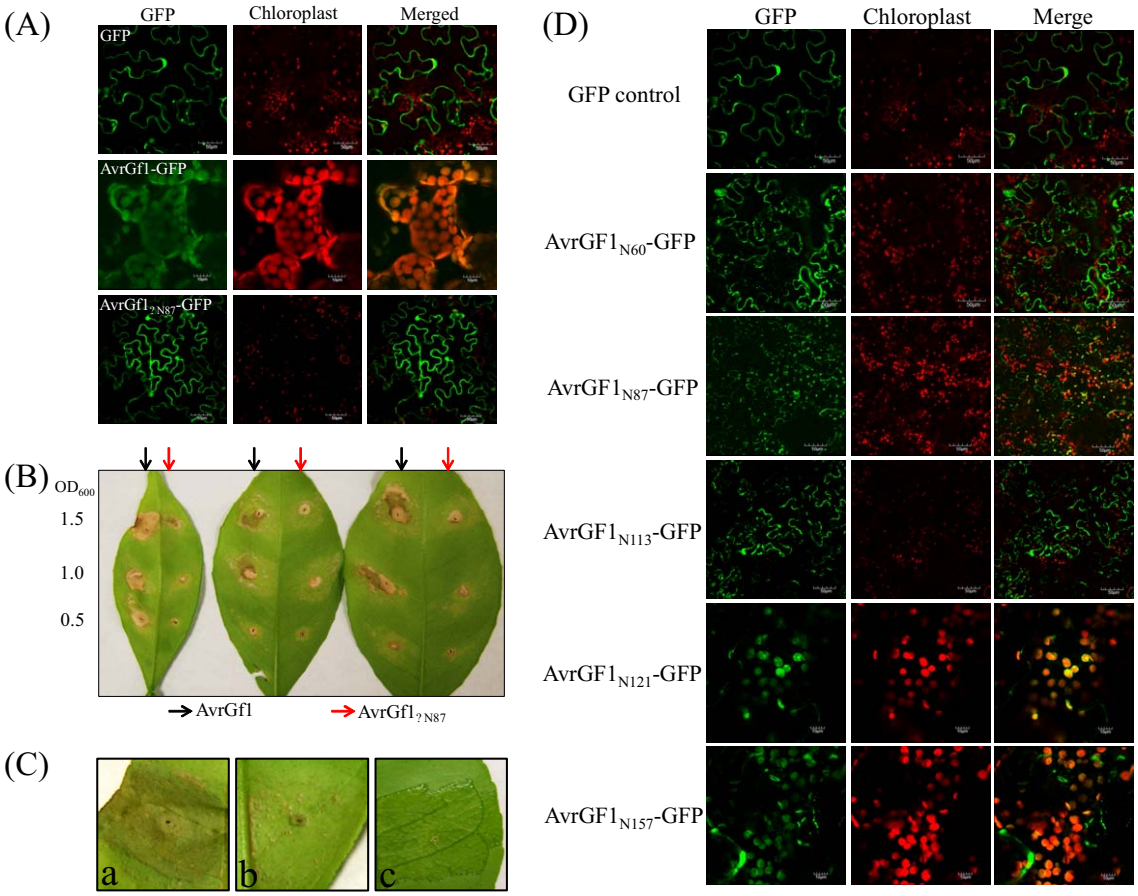

Fig. 2 Characterization of the transit peptide located at the N-terminal moiety of the AvrGF1 effector. (A) AvrGF1-GFP, AvrGf1$_{\Delta N87}$-GFP and green fluorescent protein (GFP) empty vector were transiently expressed in *Nicotiana benthamiana* leaves through *Agrobacterium*-mediated infiltration. Images were taken at 3 days post-inoculation (dpi) using confocal microscopy. (B) *Agrobacterium* strains harbouring AvrGF1 and AvrGf1ΔN87 were needlelessly infiltrated into sweet orange leaves with different optical densities (ODs) as presented. Hypersensitive response (HR) induction was observed and photographed at 3 dpi. (C) *Xanthomonas citri* strains harbouring AvrGF1, HpaA-AvrGf1 and HpaA-AvrGf1$_{\Delta N87}$ were inoculated into grapefruit leaves to test the HR. Images (a) and (b) were photographed at 2 dpi, and (c) was photographed at 5 dpi. (a) 306/p53-AvrGf1; (b) 306/p53-HpaA-AvrGf1; (c) 306/p53-HpaA-AvrGf1$_{\Delta N87}$. (D) Different lengths of amino acid residues from AvrGF1 N-terminus were fused with GFP. Chimeric proteins were transiently expressed in *N. benthamiana* leaves through *Agrobacterium*-mediated infiltration. Images were photographed at 3 dpi using confocal microscopy.

Rybak *et al.*, 2009). The XopAG homologue, *avrGf2*, which also elicits an HR in grapefruit, has been isolated from *Xcc-C* (Gochez *et al.*, 2015). A Cyp-binding site (GPLL) present in *avrGf2* (and in every member of the XopAG effector family) was essential for the expression of an HR (Gochez *et al.*, 2016). Mutagenesis of the GPLL site in *avrGf2* resulted in a loss of the ability to elicit an HR (Gochez *et al.*, 2016). Molecular modelling and *in silico* docking studies for the Cyp–*avrGf2* interaction predicted the binding of citrus Cyp, which accelerates protein folding through a peptidyl–prolyl isomerase activity that catalyses a *cis–trans* isomerization of proline (Pro) peptide bonds (Gochez *et al.*, 2016). The proposed model for the mode of action of *avrGf2* in citrus suggests an important role for Cyp as a catalyst. *AvrGf2* enters the plant cell, using the T3SS, as an inactive form; once inside the cell, it is recognized by Cyp and *cis–trans* modified (Gochez *et al.*, 2016). This conformational change in *avrGf2* thereby triggers the resistance response (Gochez *et al.*, 2016). In addition, the CTD of XopAG family effectors contains a highly conserved motif, CLNAxYD, which was identified to be crucial for the induction of HR based on site-directed mutagenesis (Gochez *et al.*, 2016).

Mutations in *hrpG*, *hrpX* or *hrpA* genes resulted in a complete loss of pathogenicity (Figueiredo *et al.*, 2011a). These mutants continued to elicit HRs in non-host tomato, indicating the presence of an *hrp*-independent elicitor of HR (Figueiredo *et al.*, 2011a). Other components of the *Xcc* Hrp cluster, *hrpB*, *hrpD* and *hrpF*, are essential for canker production in hosts and induce HR in non-hosts (Dunger *et al.*, 2005). Various protein–protein interactions have been discovered amongst the various sequenced genes of the *Xcc hrp* pathogenicity island, including between HrpG and XAC0095, between HpaA, HpaB and HrcV, between HrpB1, HrpD6 and HrpW, and between HrpB2 and HrcU (Alegria *et al.*, 2004). The two-component system of ColR/ColS in *Xcc* positively regulates the expression of *hrpD6*, *hpaF*, the LPS synthesis gene *rfbC* and *katE*, a catalase gene. ColS, a transmembrane sensor kinase, responds to an environmental stimulus and autophosphorylates; it then transfers the phosphoryl group to the cognate response regulator, ColR, which affects the regulatory effect (Yan and Wang, 2011).

The TonB-dependent receptor (TBDR), which comprises a receptor protein family, assembles in the outer membrane of Gram-negative bacteria, acts as an iron transporter and takes up iron-siderophore complexes and vitamin B12 (Aini *et al.*, 2010). The TBDR, XAC4131, does not appear to play an important role in pathogenicity; however, mutation of this gene in *Xcc* resulted in a delay in HR in tobacco (Aini *et al.*, 2010). This TBDR, XAC4131, controls the expression of *hrpG*, an *hrp* regulatory gene (Aini *et al.*, 2010). Transcription of XAC4131 is regulated by Fur protein (Aini *et al.*, 2010).

Flagellin, a highly conserved component of *Xcc* flagella, is a common pathogen-associated molecular pattern (PAMP) and an elicitor of the plant immune response (Gómez-Gómez and Boller, 2002). Within 6 h of exposure to Xflg22, the specific PAMP protein of *Xcc* encoded by *flg22*, multiple defence-related genes in kumquat are induced, whereas the same PAMP elicits no response in highly susceptible grapefruit (Shi *et al.*, 2015). *Xcc* LPSs are also recognized as PAMPs, and activate basal defence in host and non-host plants (Petrocelli *et al.*, 2012). A citrus mitogen-activated protein kinase (MAPK), CsMAPK1, appears to be another factor in the host response to infection, leading to reduced susceptibility to disease. CsMAPK1 is induced by *X. axonopodis* pv. *aurantifolii* pathotype C (*Xaa-C*) in sweet orange, a non-host, but not by *Xcc* (de Oliveira *et al.*, 2013). Artificially increased expression of CsMAPK1 in sweet orange infected with *Xcc* caused reduced canker symptoms and decreased bacterial growth (de Oliveira *et al.*, 2013).

Xcc infection results in an increase in the synthesis and mobilization of auxin and gibberellic acid (Cernadas and Benedetti, 2009). These increases lead to the up-regulation of genes associated with the host production of cellulases, pectinesterases and expansins, and the down-regulation of genes associated with the production of auxins, pectinacetyltransferases and xyloglucan galactosyltransferases (Cernadas and Benedetti, 2009). Correspondingly, auxin inhibition reduces canker severity, whereas gibberellic acid inhibition reduces auxin-dependent transcription otherwise induced by *Xcc* infection (Cernadas and Benedetti, 2009). *Xcc* uses a plant natriuretic peptide, XacPNP, to modify host homeostasis and growth (Gottig *et al.*, 2008). PNPs are a class of extracellular, systemically mobile molecules, which elicit multiple plant responses involved in homeostasis and growth, including stomatal opening, water uptake and tissue-specific ion movement (Ludidi *et al.*, 2004; Maryani *et al.*, 2001; Pharmawati *et al.*, 2001). XacPNP has no known homologue in other bacteria (Gottig *et al.*, 2008). The production of XacPNP is induced by the nutrient-poor apoplast, and this production is believed to be a mechanism by which *Xcc* modifies its host to create a more favourable environment (Gottig *et al.*, 2008). Instead of restricting nutrition to the site of infection, the plant is induced via XacPNP to continue to send resources to the affected area, which are then used as food for the multiplying *Xcc* population. XacPNP deletion mutants elicit larger areas of necrotic tissue in their host, suggesting that, in the absence of XacPNP, the host plant is better able to properly respond to infection by restricting the flow of nutrients and water to the infected area (Gottig *et al.*, 2008).

EPIDEMIOLOGY

Xcc is disseminated via wind-driven rain, and the incidence and severity of CC are exacerbated by the larval feeding of the Asian citrus leafminer (CLM; *Phyllocnistis citrella*) (Belasque *et al.*, 2005; Bock *et al.*, 2005; Gottwald *et al.*, 2007; Graham *et al.*, 2004). *Xcc* is at the greatest concentration of inoculum immediately following wetting of viable lesions (Bock *et al.*, 2005). Inoculum

concentration in rain water is lower after about an hour of rain and wind; however, bacteria reproduce quickly enough between storms for large initial concentrations to be available for each wind-driven rain event (Bock *et al.*, 2005). Temperatures between 20 and 30 °C support adequate growth of *Xcc* and, as such, tropical and subtropical environments can support large populations year round (Bock *et al.*, 2005). In addition to favourable temperatures, tropical and subtropical rain events tend to be frequent, short and produce strong gusty winds, a combination highly favourable for the spread of *Xcc* (Bock *et al.*, 2005). Higher speed winds lead to greater plant wounding, which results in more extreme infections in terms of both severity and incidence (Bock *et al.*, 2010a). Hurricanes and tornados are capable of widely dispersing inoculum (Bock *et al.*, 2010a; Gottwald and Irey, 2007). Under optimal conditions of temperature, humidity and inoculum, symptoms can manifest around 7 days after infection (Schubert *et al.*, 2001). When temperatures or inoculum levels are low, symptoms can take 2 months or more to manifest (Schubert *et al.*, 2001). When temperatures exceed 40 °C, symptoms typically do not develop on sweet orange, but develop on Tahiti lime, given sufficient leaf wetness duration (Christiano *et al.*, 2009; Dalla Pria *et al.*, 2006). Prolonged leaf wetness increases the severity of CC in sweet orange and lesion development in Tahiti lime (Christiano *et al.*, 2009; Dalla Pria *et al.*, 2006).

CC produces local erumpent lesions on young expanding leaf, stem and green fruit tissue (Schubert *et al.*, 2001). Lesions on leaves and fruit often have a water-soaked margin, surrounded by a chlorotic halo, but stem lesions do not have chlorotic haloes (Schubert *et al.*, 2001). With age, CC lesions grow in diameter and turn brown with a corky appearance (Schubert *et al.*, 2001). Eventually, leaf lesions may fall out and heavy infestation can lead to defoliation (Schubert *et al.*, 2001). *Xcc* infection is directly related to ethylene production and subsequent defoliation, which occurs more rapidly with higher inoculum concentration and with infections closer to the stipules (Dutta and Biggs, 1991; Goto *et al.*, 1980). Young canker lesions on host leaves, stems and fruit have a higher initial inoculum concentration than older lesions (Bock *et al.*, 2005).

Depending on temperature, host citrus type and initial inoculum concentration, within 1–3 weeks post-infection the host epidermis ruptures, releasing bacteria to the surface, where they can spread to initiate new infection cycles (Brunings and Gabriel, 2003). Bacterial exudates from citrus lesions contain high concentrations of biofilm-coated aggregates of *Xcc* inoculum, which serve to spread the bacteria to young exposed tissue on the same plant or on new plants (Timmer *et al.*, 1991). *Xcc* infiltrates the host via wounds and natural stomatal and hydathode openings. Tissues of citrus plants susceptible to CC show an increased tolerance to the disease with maturity (Lee, 1921). The decrease in water congestion of fully expanded, mature leaves is a major factor in their resistance to bacterial infection (Verniere *et al.*, 2003). The correlation between maturity and CC susceptibility seems to be similar for stems, fruits and leaves, with stomatal infection of these tissues being restricted to the period of expansion (Graham *et al.*, 2004; Verniere *et al.*, 2003). Although mature bark-covered stems may resist infection, those stems which are infected whilst green can maintain populations of *Xcc* for years, producing inoculum from the raised corky lesions when wetted (Schubert *et al.*, 2001). *Xcc* survives primarily and seasonally within the confines of the lesions. Outside of lesions, *Xcc* survives only 1–3 days on inanimate surfaces, such as clothing and agricultural equipment, and no more than 2 months in soil as a result of competition with saprophytes (Graham *et al.*, 1989; Schubert *et al.*, 2001). In tropical environments with mild winter temperatures, viable *Xcc* cells persist in the margins of older lesions on leaves, fruit and twigs (Pruvost *et al.*, 2002).

BIOFILM FORMATION

Among the different strains of *Xcc*, those with restricted host ranges produce more biofilm on leaves and fruits of their host than on non-hosts, indicating the importance of biofilm production as a virulence factor (Sena-Vélez *et al.*, 2015). The regulation of pathogenicity factors (*rpf*) gene cluster is responsible for the synthesis of extracellular enzymes, such as proteases, endoglucanases and polygalacturonases, which inhabit the extracellular polymeric substance (EPS) (Dunger *et al.*, 2007). The *rpf* gene cluster also regulates the production of xanthan, a secreted exopolysaccharide (Dunger *et al.*, 2007). The *Xcc* gene, *galU*, is necessary for growth in grapefruit, and is involved in the biosynthesis of xanthan, capsular polysaccharide and biofilm formation (Guo *et al.*, 2010). The *rpfG* gene specifically regulates genes involved in the synthesis of extracellular enzymes and EPS, and the formation of biofilms (Slater *et al.*, 2000). Various genes primarily involved in activities such as amino acid synthesis, energy metabolism, DNA replication and transcription, membrane transport and signal transduction, also seem to be involved in biofilm formation (Malamud *et al.*, 2013). Mutation of the T3SS resulted in changes in the expression of proteins involved in EPS production, impairment of leaf-associated growth and elimination of the ability to form a biofilm (Zimaro *et al.*, 2014).

The *rpf* gene cluster also encodes the cell–cell signalling system (Rigano *et al.*, 2007). Xanthan production in *X. campestris* is regulated by a cell–cell signalling system encoded by *rpf* genes and DSF, and evidence suggests a similar mechanism (*rpf*/DSF) within *Xcc* (Crossman and Dow, 2004; Rigano *et al.*, 2007; Siciliano *et al.*, 2006). Structurally, biofilm development is initiated by bacterial attachment to a surface, followed by the formation of bacterial aggregates called microcolonies (Rigano *et al.*, 2007). These microcolonies are tightly packed bacteria, sometimes in regular hexagonal formation, separated by channels of water (Rigano *et al.*, 2007). Xanthan and the presence of extracellular

DNA are important during the early stages of biofilm formation (Rigano *et al.*, 2007; Sena-Vélez *et al.*, 2016). The xanthan in the biofilm provides protection from both desiccation and plant antimicrobial compounds (Dow *et al.*, 2003). In terms of pathogenicity, xanthan can contribute to plant susceptibility by suppressing callose deposition (Yun *et al.*, 2006). *Xcc* grown on nutrient-rich medium shows retarded development of microcolonies, suggesting that cues for biofilm formation are dependent on the relatively nutrient-poor environment of the intercellular spaces of leaf mesophyll tissue (Rigano *et al.*, 2007). Proteome changes in mature *Xcc* colonies in a biofilm versus planktonic cells include an upregulation in the production of proteins involved in the outer membrane and receptor/transport proteins, indicating how the external biofilm structure is maintained and how molecules and signals are disseminated (Zimaro *et al.*, 2013).

Within the *rpf* gene cluster is the *gum* cluster, which is composed of 12 ORFs and is 98% conserved across the plant-pathogenic xanthomonads (Dunger *et al.*, 2007). The *Xcc* glycotransferase gene, *gumD*, is involved in the synthesis of the first xanthan lipid intermediate and is an essential component of biofilm formation (Rigano *et al.*, 2007). Deletion mutants of *gumD* in *Xcc* remove the ability to produce xanthan (Dunger *et al.*, 2007). However, *gumD* is not necessary for virulence, as *gumD* mutants of *Xcc* still multiply at the same rate as the wild-type, still elicit HR in non-hosts and remain pathogenic (Dunger *et al.*, 2007). However, impairment of biofilm formation in *Xcc* reduces its survival rate whilst under oxidative stress in the stationary growth phase (*Xcc* is naturally susceptible to oxidative stress during the exponential growth phase when no EPS is produced), as well as reducing its epiphytic growth and survival on leaves (Dunger *et al.*, 2007; Rigano *et al.*, 2007). Another gene in the *gum* cluster is *gumB*, which encodes a protein that polymerizes a pentasaccharide intermediate into mature xanthan (Vojnov *et al.*, 1998). Experiments on *gumB* mutants revealed that, although *Xcc* remained pathogenic, it manifested reduced symptoms within its host (Rigano *et al.*, 2007). Various mutants which show deficiency in biofilm formation, including *gumB* mutants, also show an impairment in motility, chemotaxis and virulence (Malamud *et al.*, 2013).

Eventually during disease development it is necessary for the biofilm to be detached and disaggregated in order to facilitate the dispersal of *Xcc*. Nutrient deprivation, the transition to stationary phase growth or population density, or a combination of some or all of these factors, plus possibly as yet undiscovered factors, might drive detachment (Lamed and Bayer, 1986; O'Toole *et al.*, 2000; Stoodley *et al.*, 2002). The DSF endo-β-1,4-mannanase promotes the transition from aggregate to planktonic lifestyle (Dow *et al.*, 2003).

MANAGEMENT OF CC

In regions in which CC has not yet become endemic, measures of control are focused on the isolation and eradication of the pathogen, minimization of dissemination, reduction of sources of inoculum and protection of susceptible tissues from infection (Behlau *et al.*, 2016; Civerolo, 1981; Leite and Mohan, 1990). In locations in which CC has become endemic and eradication is no longer feasible, measures for the management of the disease are adopted to avoid or reduce crop loss. A CC integrated management programme involves the planting of CC-free nursery stock, choice of less susceptible citrus cultivars, deployment of arboreal windbreaks, spraying with copper-based bactericides, control of CLM and application of systemic acquired resistance (SAR) inducers (Behlau and Belasque, 2014; Behlau *et al.*, 2008, 2010b; Gottwald and Timmer, 1995; Gottwald *et al.*, 2002; Graham and Myers, 2009, 2013; Graham *et al.*, 2010, 2011; Leite and Mohan, 1990; Stein *et al.*, 2007).

In addition to avoiding long-distance *Xcc* dissemination, the establishment of new citrus plantings with CC-free trees is important to postpone and reduce the severity of outbreaks. After planting in the field, infected nursery trees serve as sources of inoculum, which is released from the pre-existing lesions in the presence of water and transported to the surrounding trees by wind during rainstorms (Bock *et al.*, 2010a; Danos *et al.*, 1984; Gottwald *et al.*, 1992). However, because immature plant tissue of citrus trees is more susceptible to CC, delaying the establishment of the disease in orchards within endemic areas may significantly reduce its impact (Gottwald and Graham, 1992). Newly planted trees are more susceptible because leaf flush production is more frequent and represents a higher proportion of the canopy volume relative to more mature trees (Behlau *et al.*, 2010a; Leite and Mohan, 1990). Moreover, CLM feeds on young leaf tissues, and therefore contributes to increased *Xcc* severity in young groves (Christiano *et al.*, 2007; Hall *et al.*, 2010; Jesus *et al.*, 2006).

Together with copper sprays, windbreaks are highly effective for the management of CC (Gottwald and Timmer, 1995; Leite and Mohan, 1990; Moschini *et al.*, 2014). This control measure reduces the direct interaction of wind with trees, providing less favourable conditions for the spread and penetration of bacteria into the host. The arboreal barrier also lessens the damage caused by high-speed winds, reducing entry wounds for the bacterium. In Argentina, measured levels of CC on trees protected by windbreaks were lower than those on trees treated with copper sprays (Gottwald and Timmer, 1995).

The spray application of copper-based bactericides is a key measure for CC management. Copper is usually applied during the spring and summer months, when immature plant tissue is abundant and climatic conditions are most favourable to the pathogen. This measure is strictly preventative with no curative or systemic activity, as copper acts by reducing inoculum buildup on new leaf flush and protecting expanding fruit and leaf surfaces from infection (Behlau *et al.*, 2008, 2010b; Graham *et al.*, 2010,

2011, 2016a). Fixed or insoluble forms of copper, such as copper hydroxide, copper oxychloride and copper oxide, are the formulations most widely used. Because copper ions are slowly released, fixed copper is less phytotoxic to plants and provides better residual activity against *Xcc* than can be achieved with non-fixed copper. After being sprayed, an insoluble copper film is formed on the treated surface, which prevents new infections by reducing the viability of *Xcc* accessing the susceptible plant tissue. Copper ions gradually released from the fixed forms confer chemical protection by killing the bacterium on arrival on the treated surface (Graham *et al.*, 2010; Menkissoglu and Lindow, 1991). The rate of metallic copper used and the frequency of spray applications per season depend on the weather conditions and the desired period of protection (Behlau *et al.*, 2010b, 2017b). About 0.54–1.12 kg of metallic copper per hectare every 21 days is recommended to protect spring flush growth or fruit surfaces (Behlau *et al.*, 2017b; Dewdney *et al.*, 2016; Scapin *et al.*, 2015).

CC management on susceptible citrus cultivars is challenging when exclusively using copper bactericides, because wind-blown rain introduces *Xcc* directly into stomata, bypassing the protective copper film on the plant surface (Behlau *et al.*, 2010b; Bock *et al.*, 2010b; Graham and Myers, 2013; Leite and Mohan, 1990; Stein *et al.*, 2007). Therefore, when conditions are conducive to CC outbreaks, the combined use of copper sprays and windbreaks is mandatory to successfully control the disease. Even with windbreaks, frequent re-applications of copper are required to protect fruits that are continuously expanding over a 90–120-day timeframe, depending on the citrus cultivar (Behlau *et al.*, 2010b; Stall *et al.*, 1982; Stein *et al.*, 2007). Although copper sprays greatly contribute to CC control, they should be used with caution. Sprayed copper accumulates in the soil, where it may negatively affect root growth and nutrient uptake by the citrus trees (Alva *et al.*, 1995; Fan *et al.*, 2011; Graham *et al.*, 1986). In addition, high copper concentrations may lead to fruit blemishing as a result of phytotoxicity, and the frequent use of copper in citrus orchards favours the development of copper-resistant strains in xanthomonad populations, which may impair future disease management with these bactericides (Albrigo *et al.*, 1997; Behlau *et al.*, 2011, 2013; Canteros *et al.*, 2010; Graham *et al.*, 2007).

CC severity and incidence increase in the presence of CLM, and lesions coincident with CLM-induced leaf damage are larger, denser and produce more inoculum than stomatal infections (Gottwald *et al.*, 2007; Graham *et al.*, 2004). CLMs feed and rapidly reproduce on vigorous flushes of young citrus trees with the potential for explosive increases in *Xcc* inoculum (Gottwald, 2010; Stein *et al.*, 2007). Mated females oviposit on expanding leaves, and emerging larvae tunnel under the leaf cuticle to feed. CLM leaf damage by tunnelling increases the host's vulnerability to CC by creating wounds which expose the inner mesophyll tissue to infection (Graham *et al.*, 2004). CLM infests all types of citrus and other members of the Rutaceae family, but its preferred hosts on which to feed and oviposit are grapefruit, tangerine and pumelo (Stelinski *et al.*, 2010). Control of the mining larvae is mainly performed by the application of abamectin and neonicotinoid insecticides (Powell *et al.*, 2007; Stein *et al.*, 2007). In previous studies, soil applications of neonicotinoids were highly effective for reducing foliar infection and CC-induced defoliation on non-bearing grapefruit trees (Graham and Myers, 2009, 2013). Systemic neonicotinoid insecticides, such as imidacloprid, thiamethoxam and clothianidin, can be applied year-round as soil drenches to non-bearing citrus trees for the control of CLM and the associated infection of leafminer galleries by *Xcc* (Rogers *et al.*, 2015). Soil-applied neonicotinoid insecticides are used in young orchards (up to 3 years), whereas abamectin may be sprayed on the foliage of trees of all ages. Foliar-applied insecticides provide a shorter period of protection relative to soil applications and are performed during summer and autumn to protect developing leaves when the first CLM mined leaf galleries are observed, usually at the feather leaf stage. Neonicotinoids take longer to reach the canopy, and these insecticides should be applied about 2 weeks prior to leaf expansion in order to be available in the flushes when the CLM larvae begin to feed (Rogers, 2012; Rogers *et al.*, 2015).

Biological control of CLM has limited efficiency. The best-known and studied natural enemy of CLM is the wasp *Ageniaspis citricola* and, although this predator may show high parasitism rates, its deployment has not been demonstrated to be an equivalently effective alternative to the use of chemical sprays, especially when there are high infestations of CLM (Hoy and Jessey, 2004; Johnson and Henne, 2003). CLM is parasitized by numerous others organisms; however, these parasites are often eradicated by pesticides applied to combat CLM itself (Elekcioglu, 2013; Mafi and Ohbayashi, 2010; Pena *et al.*, 2000).

There have been successful attempts to control CLM by disruption of mating with artificially synthesized pheromones which behave either as a competitive disruptor (an attractant) or as a non-competitive disruptor, which includes substances that either desensitize the insect to its natural pheromone or confuse the insect by overloading the environment with the pheromone, a component of the pheromone or a synthetic mimic (Miller *et al.*, 2006; Stelinski *et al.*, 2008, 2010). Disruption of CLM flush infestation was significantly reduced by the application of only 1.5 g of the active ingredient (a 3 : 1 blend of (*Z,Z,E*)-7,11,13-hexadecatrienal–(*Z,Z*)-7,11-hexadecadienal) per hectare of citrus grove twice in a 221-day investigation (Stelinski *et al.*, 2008). The same blend mixed with the insecticide permethrin killed 100% of all CLM which came into contact with it (Stelinski and Czokajlo, 2010).

SAR is a natural plant defence that provides long-lasting protection against a broad spectrum of microorganisms (An and Mou, 2011; Fu and Dong, 2013). SAR requires the signal molecule

salicylic acid (SA) and is associated with the accumulation of pathogenesis-related (PR) proteins, which are thought to contribute to resistance (Zhang YX *et al.*, 2010). SAR may be activated in the absence of pathogens by treating plants with chemical inducers (Gorlach *et al.*, 1996). Acibenzolar-*S*-methyl (ASM), a functional homologue of SA, is the most widely known commercially produced inducer of SAR (Tally *et al.*, 1999). In glasshouse trials, soil drenches of ASM, as well as neonicotinoids, induced a high and persistent up-regulation of PR gene expression that was correlated with a reduction in CC lesions for up to 24 weeks after treatment (Francis *et al.*, 2009). Furthermore, SAR inducers demonstrated the potential to augment CC control with copper sprays (Graham and Myers, 2013). ASM provides a non-insecticidal option for sustaining SAR activity in trees greater than 3 m in height with low risk of movement through the root zone into the soil profile. Thus, soil-applied ASM provides an option for delivering the SAR compound to fruiting trees and has been shown to increase the efficacy of copper spray programmes when used in tandem (Graham and Myers, 2013). Integration of ASM may also enable a reduction in the rate and frequency of copper sprays and potentially mitigate the risk of copper resistance development. Integration of ASM with copper has been demonstrated to protect crops in which copper-resistant pathogen strains predominate, as was reported for the management of bacterial speck and bacterial spot of tomato (Louws *et al.*, 2001). Other chemicals that have been successfully tested but, for different reasons, are not widely used for CC control are streptomycin and zinc oxide (Behlau *et al.*, 2012b; Graham *et al.*, 2007, 2010, 2016b).

Although copper-based bactericides have proven to be effective for the control of CC, these chemicals are unable to completely suppress the inoculum, and frequent applications throughout the spring and summer are required to minimize losses (Behlau *et al.*, 2008, 2010b, 2017b; Graham *et al.*, 2010, 2011, 2016a; Scapin *et al.*, 2015). The pressure imposed by the continuous application of copper drives the selection of resistant strains and favours a gradual increase in the frequency of resistant pathogens within the bacterial population, which jeopardizes the continued effectiveness of the sprays (Sundin *et al.*, 1989). *Xcc* strains resistant to copper (Cu^R) were detected in Argentina in the mid-1990s (Canteros *et al.*, 2010). Genetic characterization of copper resistance determinants in these strains revealed that the resistance is mainly caused by the plasmid-borne gene cluster *cop*LAB, which is also present in strains of Cu^R *Xanthomonas alfalfae* ssp. *citrumelonis* (*Xac*) (Behlau *et al.*, 2011).

Horizontal transfer of copper resistance determinants by conjugation is the primary mechanism for the acquisition of copper resistance by bacteria, including *Xcc* and *Xac* (Behlau *et al.*, 2012a). Copper resistance in bacteria is conferred by several genes normally organized in operons; therefore, a natural spontaneous mutation conferring copper resistance is unlikely to occur within bacterial populations (Mellano and Cooksey, 1988). Previous findings have indicated that copper sequestration and accumulation are the primary copper resistance mechanisms in *Xanthomonas*, and *copL* regulates the expression of *copA* and *copB*, which encode for copper-binding proteins (Cooksey, 1990; Voloudakis *et al.*, 2005).

Comparison of the partial nucleotide sequences of *copLAB* from Cu^R *Xcc* with other species of Cu^R *Xanthomonas* revealed that the *copLAB* cluster is present in many species of *Xanthomonas* from different parts of the world, and multiple independent introductions of resistance genes have occurred in resistant populations of *Xcc* in Argentina and *Xac* in Florida, USA (Behlau *et al.*, 2013). The alignment of partial sequences of resistance genes among strains of Cu^R *Xanthomonas* revealed homology of $\geq$92%, $\geq$96% and $\geq$91% for *copL*, *copA* and *copB*, respectively. The presence of *copLAB* homologues was also observed in epiphytic bacteria, such as non-pathogenic xanthomonads and a *Stenotrophomonas maltophilia* strain isolated from citrus (Behlau *et al.*, 2012a). *cop* genes have been mistakenly annotated and studied as putative copper resistance genes for several xanthomonads (Hsiao *et al.*, 2011; da Silva *et al.*, 2002; Teixeira *et al.*, 2008). This misattribution is mainly because *cohLAB* genes, homologues of *cop* genes, are present on the chromosomes of both copper-sensitive (Cu^S) and Cu^R xanthomonads (Behlau *et al.*, 2011, 2013). Both gene groups have been shown to be responsive to copper; however, *coh* genes are not responsible for copper resistance, but are probably necessary for homeostasis or copper metabolism (Behlau *et al.*, 2011; Hsiao *et al.*, 2011; Teixeira *et al.*, 2008). Although Cu^R *Xanthomonas* strains grow on MGY agar amended with copper sulfate at 400 mg/L, Cu^S strains can continue to grow only up to copper concentrations of 75–150 mg/L (Behlau *et al.*, 2012a, 2013, 2017a). More recently, copper tolerant (Cu^T) strains of *Xanthomonas* have been identified. These strains can grow at intermediate concentrations of copper sulfate and are differentiated from the Cu^R strains by their lack of the copper resistance cluster *copLAB*, and from the Cu^S strains by a greater expression of *cohLAB* in the presence of copper (F. Behlau, 2016).

In addition to breeding commercial citrus varieties with less CC-susceptible citrus types and relatives, other attempts have been made to defend against *Xcc* with varying degrees of success. Copper biocides are a standard method of control; however, the ability of *Xcc* to acquire plasmid-borne genes conferring resistance to copper sprays greatly limits their long-term use. D-Leucine and 3-indolylacetonitrile (IAN) were found to inhibit *Xcc* from producing a biofilm and increased the bacterium's susceptibility to copper ($CuSO_4$) treatments; furthermore, IAN repressed the expression of genes involved in chemotaxis and motility (Li and Wang, 2014). Phage treatment of *Xcc* has some degree of efficacy for moderately sensitive Valencia oranges (about 41% disease

reduction), but has proven to be ineffective on grapefruit, a more susceptible host (Balogh *et al.*, 2008).

Transient expression of *Bs2* (an *R* gene from pepper, which provides resistance to *X. campestris* pv. *vesicatoria*) in citrus has been reported to lead to decreased susceptibility to CC (Sendin *et al.*, 2012). Likewise, transgenic citrus with the rice *Xa21 R* gene showed decreased susceptibility to CC (Mendes *et al.*, 2010). In addition, transgenic grapefruit and sweet orange expressing the Arabidopsis *NPR1* gene (*AtNPR1*), a positive regulator of SAR, were less susceptible to CC (Zhang XD *et al.*, 2010). The application of ASM for the induction of SAR has also shown effectiveness in reducing the incidence and severity of CC (Graham and Myers, 2013). Control of *Xcc* via foliar applications of chemicals which trigger the host plant's induced systemic resistance do not seem particularly effective on their own (Graham and Leite, 2004). SA foliar treatments increase endogenous SA and reduce lesion incidence and size (Wang and Liu, 2012).

Clustered Regularly Interspace Short Palindromic Repeats technology using the Cas9 enzyme and guide RNA has shown promise in generating canker-resistant citrus varieties by modifying the PthA4 effector-binding elements (EBEs) in the promoter or coding region of the *CsLOB1* gene (Jia *et al.*, 2016, 2017). Genome editing of the disease susceptibility gene *CsLOB1* in Duncan grapefruit confers resistance to CC, which might provide a long-term solution to control CC if non-transgenic EBE_{PthA4}- or *CsLOB1*-modified plants are produced successfully without negative effects on other horticultural traits (Jia *et al.*, 2017).

REFERENCES

Aini, L.Q., Hirata, H. and Tsuyumu, S. (2010) A TonB-dependent transducer is responsible for regulation of pathogenicity-related genes in *Xanthomonas axonopodis* pv. *citri*. *J. Gen. Plant Pathol.* **76**, 132–142.

Albrigo, L.G., Timmer, L.W., Townsend, K. and Beck, H.W. (1997) Copper fungicides-residues for disease control and potential for spray burn. *Proc. Florida State Hortic. Soc.* **110**, 67–70.

Alegria, M.C., Docena, C., Khater, L., Ramos, C.H.I., da Silva, A.C.R. and Farah, C.S. (2004) New protein–protein interactions identified for the regulatory and structural components and substrates of the type III secretion system of the phytopathogen *Xanthomonas axonopodis* pathovar *citri*. *J. Bacteriol.* **186**, 6186–6197.

Al-Saadi, A., Reddy, J.D., Duan, Y.P., Brunings, A.M., Yuan, Q.P. and Gabriel, D.W. (2007) All five host-range variants of *Xanthomonas citri* carry one pthA homolog with 17.5 repeats on citrus, but none determine host-range pathogenicity variation. *Mol. Plant–Microbe Interact.* **20**, 934–943.

Al-Saleh, M.A., Widyawan, A., Saleh, A.A. and Ibrahim, Y.E. (2014) Distribution and pathotype identification of *Xanthomonas citri* subsp. *citri* recovered from southwestern region of Saudi Arabia. *Afr. J. Microbiol. Res.* **8**, 673–679.

Alva, A.K., Graham, J.H. and Anderson, C.A. (1995) Soil-pH and copper effects on young Hamlin orange trees. *Soil. Sci. Soc. Am. J.* **59**, 481–487.

Alvarez, A.M., Benedict, A.A., Mizumoto, C.Y., Pollard, L.W. and Civerolo, E.L. (1991) Analysis of *Xanthomonas campestris* pv *citri* and *X. c. citrumelo* with monoclonal-antibodies. *Phytopathology*, **81**, 857–865.

An, C.F. and Mou, Z.L. (2011) Salicylic acid and its function in plant immunity. *J. Integr. Plant Biol.* **53**, 412–428.

Balogh, B., Canteros, B.I., Stall, K.E. and Jones, J.B. (2008) Control of citrus canker and citrus bacterial spot with bacteriophages. *Plant Dis.* **92**, 1048–1052.

Behlau, F. and Belasque, J. (2014) *Cancro Citrico – A Doenca e Seu Controle*. Araraquara, Brazil: Fundecitrus.

Behlau, F., Belasque, J., Bergamin, A., Graham, J.H., Leite, R.P. and Gottwald, T.R. (2008) Copper sprays and windbreaks for control of citrus canker on young orange trees in southern Brazil. *Crop Prot.* **27**, 807–813.

Behlau, F., Amorim, L., Belasque, J., Bergamin, A., Leite, R.P. and Graham, J.H. and Gottwald, T.R. (2010a) Annual and polyetic progression of citrus canker on trees protected with copper sprays. *Plant Pathol.* **59**, 1031–1036.

Behlau, F., Belasque, J., Graham, J.H. and Leite, R.P. (2010b) Effect of frequency of copper applications on control of citrus canker and the yield of young bearing sweet orange trees. *Crop Prot.* **29**, 300–305.

Behlau, F., Canteros, B.I., Minsavage, G.V., Jones, J.B. and Graham, J.H. (2011) Molecular characterization of copper resistance genes from *Xanthomonas citri* subsp *citri* and *Xanthomonas alfalfae* subsp *citrumelonis*. *Appl. Environ. Microb.* **77**, 4089–4096.

Behlau, F., Canteros, B.I., Jones, J.B. and Graham, J.H. (2012a) Copper resistance genes from different xanthomonads and citrus epiphytic bacteria confer resistance to *Xanthomonas citri* subsp *citri*. *Eur. J. Plant Pathol.* **133**, 949–963.

Behlau, F., Jones, J.B., Myers, M.E. and Graham, J.H. (2012b) Monitoring for resistant populations of *Xanthomonas citri* subsp *citri* and epiphytic bacteria on citrus trees treated with copper or streptomycin using a new semi-selective medium. *Eur. J. Plant Pathol.* **132**, 259–270.

Behlau, F., Hong, J.C., Jones, J.B. and Graham, J.H. (2013) Evidence for acquisition of copper reistance genes from different sources in citrus-associated xanthomonads. *Phytopathology*, **103**, 409–418.

Behlau, F., Fonesca, A.E. and Belasque, J. Jr. (2016) A comprehensive analysis of the citrus eradication program in Sao Paulo State from 1999 to 2009. *Plant Pathol.* **65**, 1390–1399.

Behlau, F., Gochez, A., Lugo, A., Elibox, W., Minsavage, G., Potnis, N., White, F., Ebrahim, M., Jones, J. and Ramsubhag, A. (2017a) Characterization of a unique copper resistance gene cluster in *Xanthomonas campestris* pv. *campestris* isolated in Trinidad, West Indies. *Eur. J. Plant Pathol.* **147**, 671–681.

Behlau, F., Scandelai, L., Junior, G. and Lanza, F. (2017b) Soluble and insoluble copper formulations and metallic copper rate for control of citrus canker on sweet orange trees. *Crop Prot.* **94**, 185–191.

Belasque, J., Parra-Pedrazzoli, A.L., Rodrigues Neto, J., Yamamoto, P.T., Chagas, M.C.M., Parra, J.R.P., Vinyard, B.T. and Hartung, J.S. (2005) Adult citrus leadminers (*Phyllocnistis citrella*) are not efficient vectors for *Xanthomonas axonopodis* pv. *citri*. *Plant Dis.* **89**, 590–594.

Bock, C.H., Parker, P.E. and Gottwald, T.R. (2005) Effect of simulated wind-driven rain on duration and distance of dispersal of *Xanthomonas axonopodis* pv. *citri* from canker-infected citrus trees. *Plant Dis.* **89**, 71–80.

Bock, C.H., Graham, J.H., Gottwald, T.R., Cook, A.Z. and Parker, P.E. (2010a) Wind speed and wind-associated leaf injury affect severity of citrus canker on Swingle citrumelo. *Eur. J. Plant Pathol.* **128**, 21–38.

Bock, C.H., Graham, J.H., Gottwald, T.R., Cook, A.Z. and Parker, P.E. (2010b) Wind speed effects on the quantity of *Xanthomonas citri* subsp. *citri* dispersed downwind from canopies of grapefruit trees infected with citrus canker. *Plant Dis.* **94**, 725–736.

Bouzar, H., Jones, J.B., Stall, R.E., Hodge, N.C., Minsavage, G.V., Benedict, A.A and Alvarez, A.M. (1994) Physiological, chemical, serological, and pathogenic analyses of a worldwide collection of *Xanthomonas campestris* pv *vesicatoria* strains. *Phytopathology*, **84**, 663–671.

Brunings, A.M. and Gabriel, D.W. (2003) *Xanthomonas citri*: breaking the surface. *Mol. Plant Pathol.* **4**, 141–157.

Canteros, B.I., Gochez, A.M., Rybak, M.A., Minsavage, G.V., Jones, J.B. and Stall, R.E. (2010) Management and characterization of plasmid-encoded copper resistance in *Xanthomonas axonopodis* pv. *citri*. p. 145. *International Conference on Plant Pathogenic Bacteria*. 12. ICPPB. 2010 06 07–11, June 7–11, 2010. Saint-Denis, France. FR.

Cernadas, R.A. and Benedetti, C.E. (2009) Role of auxin and gibberellin in citrus canker development and in the transcriptional control of cell-wall remodeling genes modulated by *Xanthomonas axonopodis* pv. *citri*. *Plant Sci.* **177**, 190–195.

Christiano, R.S.C., Dalla Pria, M., Jesus, W.C., Parra, J.R.P., Amorim, L. and Bergamin, A. (2007) Effect of citrus leaf-miner damage, mechanical damage and inoculum concentration on severity of symptoms of Asiatic citrus canker in Tahiti lime. *Crop Prot.* **26**, 59–65.

Christiano, R.S.C., Dalla Pria, M., Jesus, W.C., Amorim, L. and Bergamin, A. (2009) Modelling the progress of Asiatic citrus canker on Tahiti lime in relation to temperature and leaf wetness. *Eur. J. Plant Pathol.* **124**, 1–7.

Civerolo, E.L. (1981) Citrus bacterial canker disease: An overview. *Proc. Intn. Soc. Citric.* **1**, 390–394.

Civerolo, E.L. and Fan, F. (1982) *Xanthomonas campestris* pv *citri* detection and identification by enzyme-linked immunosorbent-assay. *Plant Dis.* **66**, 231–236.

Coletta-Filho, H.D., Takita, M.A., Souza, A.A., Neto, J.R., Destefano, S.A.L., Hartung, J.S. and Machado, M.A. (2006) Primers based on the rpf gene region provide improved detection of *Xanthomonas axonopodis* pv. *citri* in naturally and artificially infected citrus plants. *J. Appl. Microbiol.* **100**, 279–285.

Constantin, E.C., Cleenwerck, I., Maes, M., Baeyen, S., Van Malderghem, C., De Vos, P. and Cottyn, B. (2016) Genetic characterization of strains named as *Xanthomonas axonopodis* pv. *dieffenbachiae* leads to a taxonomic revision of the *X. axonopodis* species complex. *Plant Pathol.* **65**, 792–806.

Cooksey, D.A. (1990) Genetics of bactericide resistance in plant pathogenic bacteria. *Annu. Rev. Phytopathol.* **28**, 201–219.

Costerton, J.W., Lewandowski, Z., Caldwell, D.E., Korber, D.R. and Lappin-Scott, H.M. (1995) Microbial biofilms. *Annu. Rev. Microbiol.* **49**, 711–745.

Crossman, L. and Dow, J.M. (2004) Biofilm formation and dispersal in *Xanthomonas campestris*. *Microbes Infect.* **6**, 623–629.

Cubero, J. and Graham, J.H. (2002) Genetic relationship among worldwide strains of *Xanthomonas* causing canker in citrus species and design of new primers for their identification by PCR. *Appl. Environ. Microbiol.* **68**, 1257–1264.

Cubero, J. and Graham, J.H. (2004) The leucine-responsive regulatory protein (lrp) gene for characterization of the relationship among *Xanthomonas* species. *Int. J. Syst. Evol. Microbiol.* **54**, 429–437.

Cubero, J. and Graham, J.H. (2005) Quantitative real-time polymerase chain reaction for bacterial enumeration and allelic discrimination to differentiate *Xanthomonas* strains on citrus. *Phytopathology*, **95**, 1333–1340.

Dalla Pria, M., Christiano, R.C.S., Furtado, E.L., Amorim, L. and Bergamin, A. (2006) Effect of temperature and leaf wetness duration on infection of sweet oranges by Asiatic citrus canker. *Plant Pathol.* **55**, 657–663.

Danos, E., Berger, R.D. and Stall, R.E. (1984) Temporal and spatial spread of citrus canker within groves. *Phytopathology*, **74**, 904–908.

Dewdney, M., Graham, J.H. and Rogers, M.E. (2016) *Citrus Canker. Florida Citrus Pest Management Guide, SP-43*, 93–96. Gainesville, FL: University of Florida.

Doidge, E.M. (1916) The Origin and Cause of Citrus Canker in South Africa. *Union So. Africa Dept. Agr. Sei. Bul.* **8**, 20.

Domingues, M.N., de Campos, B.M., de Oliveira, M.L.P., de Mello, U.Q. and Benedetti, C.E. (2012) TAL effectors target the c-terminal domain of RNA polymerase II (CTD) by inhibiting the prolyl-isomerase activity of a CTD-associated cyclophilin. *PLoS One*, **7**, e41553.

Dow, J.M., Crossman, L., Findlay, K., He, Y.Q., Feng, J.X. and Tang, J.L. (2003) Biofilm dispersal in *Xanthomonas campestris* is controlled by cell–cell signaling and is required for full virulence to plants. *Proc. Natl. Acad. Sci. USA*, **100**, 10 995–11 000.

Dowson, W.J. (1939) On the systematic position and generic names of the gram negative bacterial plant pathogens. *Zentr. Bakteriol. Parasitenk. Abt. II.* **100**, 177–193.

Duan, Y.P., Castaneda, A., Zhao, G., Erdos, G. and Gabriel, D.W. (1999) Expression of a single, host-specific, bacterial pathogenicity gene in plant cells elicits division, enlargement, and cell death. *Mol. Plant–Microbe Interact.* **12**, 556–560.

Dunger, G., Arabolaza, A.L., Gottig, N., Orellano, E.G. and Ottado, J. (2005) Participation of *Xanthomonas axonopodis* pv. *citri* hrp cluster in citrus canker and nonhost plant responses. *Plant Pathol.* **54**, 781–788.

Dunger, G., Relling, V.M., Tondo, M.L., Barreras, M., Ielpi, L., Orellano, E.G. and Ottado, J. (2007) Xanthan is not essential for pathogenicity in citrus canker but contributes to *Xanthomonas* epiphytic survival. *Arch. Microbiol.* **188**, 127–135.

Dutta, S. and Biggs, R.H. (1991) Regulation of ethylene biosynthesis in citrus leaves infected with *Xanthomonas campestris* pv *citri*. *Physiol. Plant.* **82**, 225–230.

Egel, D.S., Graham, J.H. and Stall, R.E. (1991) Genomic relatedness of *Xanthomonas campestris* strains causing diseases of citrus. *Appl. Environ. Microbiol.* **57**, 2724–2730.

Elekcioglu, N.Z. (2013) Host–parasitoid relations between citrus leafminer, *Phyllocnistis citrella* Stainton (Lepidoptera: *Gracillariidae*) and its parasitoid *Citrostichus phyllocnistoides* Narayanan (Hymenoptera: *Eulophidae*). *Turk. Entomol. Derg. Tu.* **37**, 503–512.

El Yacoubi, B., Brunings, A.M., Yuan, Q., Shankar, S. and Gabriel, D.W. (2007) *In planta* horizontal transfer of a major pathogenicity effector gene. *Appl. Environ. Microbiol.* **73**, 1612–1621.

Escalon, A., Javegny, S., Vernière, C., Noël, L.D., Vital, K. and Poussier, S. (2013) Variations in type III effector repertoires, pathological phenotypes and host range of *Xanthomonas citri* pv. *citri* pathotypes. *Mol. Plant Pathol.* **14**, 483–496.

Euzeby, J. (2007) List of new names and new combinations previously effectively, but no validly, published, list. *Int. J. Syst. Evol. Microbiol.* **57**, 893–897.

Fan, J.H., He, Z.L., Ma, L.N.Q. and Stoffella, P.J. (2011) Accumulation and availability of copper in citrus grove soils as affected by fungicide application. *J. Soil Sediment.* **11**, 639–648.

Figueiredo, J.F.L., Minsavage, G.V., Graham, J.H., White, F.F. and Jones, J.B. (2011a) Mutational analysis of type III effector genes from *Xanthomonas citri* subsp *citri*. *Eur. J. Plant Pathol.* **130**, 339–347.

Figueiredo, J.F.L., Romer, P., Lahaye, T., Graham, J.H., White, F.F. and Jones, J.B. (2011b) Agrobacterium-mediated transient expression in citrus leaves: a rapid tool for gene expression and functional gene assay. *Plant Cell Rep.* **30**, 1339–1345.

Francis, M.I., Redondo, A., Burns, J.K. and Graham, J.H. (2009) Soil application of imidacloprid and related SAR-inducing compounds produces effective and persistent control of citrus canker. *Eur. J. Plant Pathol.* **124**, 283–292.

Fu, Z.Q. and Dong, X.N. (2013) Systemic acquired resistance: turning local infection into global defense. *Annu. Rev. Plant Biol.* **64**, 839–863.

Gabriel, D.W., Kingsley, M.T., Hunter, J.E. and Gottwald, T. (1989) Reinstatement of *Xanthomonas citri* (ex Hasse) and *Xanthomonas phaseoli* (ex Smith) to species and reclassification of all *Xanthomonas campestris* pv *citri* strains. *Int. J. Syst. Bacteriol.* **39**, 14–22.

Gochez, A., Minsavage, G.V., Potnis, N., Canteros, B.I., Stall, R.E. and Jones, J. (2015) A functional xopAG homologue in *Xanthomonas fuscans* pv. *aurantifolii* strain C limits host range. *Plant Pathol.* **64**, 1–8.

Gochez, A.M., Shantharaj, D., Potnis, N., Zhou, X., Minsavage, G.V., White, F.F, Wang, N., Hurlbert, J.C. and Jones, J.B. (2016) Molecular characterization of xopAG effector-avrGf2 from *Xanthomonas fuscans* subsp. *aurantifolii* in grapefruit. *Mol. Plant Pathol.* **18**, 405–419.

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

Gómez-Gómez, L. and Boller, T. (2002) Flagellin perception: a paradigm for innate immunity. *Trends Plant Sci.* **7**, 251–256.

Gordon, J.L., Lefeuvre, P., Escalon, A., Barbe, V., Cruveiller, S., Gagnevin, L. and Pruvost, O. (2015) Comparative genomics of 43 strains of *Xanthomonas citri* pv. *citri* reveals the evolutionary events giving rise to pathotypes with different host ranges. *BMC Genomics*, **16**, 1098.

Gorlach, J., Volrath, S., Knauf-Beiter, G., Hengy, G., Beckhove, U., Kogel, K.-H., Oostendorp, M., Staub, T., Ward, E., Kessmann, H. and Ryals, J. (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell*, **8**, 629–643.

Goto, M., Yaguchi, Y. and Hyodo, H. (1980) Ethylene production in citrus leaves infected with *Xanthomonas citri* and its relation to defoliation. *Physiol. Plant Pathol.* **16**, 343–350.

Gottig, N., Garavaglia, B.S., Daurelio, L.D., Valentine, A., Gehring, C., Orellano, E.G. and Ottado, J. (2008) *Xanthomonas axonopodis* pv. *citri* uses a plant natriuretic peptide-like protein to modify host homeostasis. *Proc. Natl. Acad. Sci. USA*, **105**, 18 631–18 636.

Gottwald, T.R. (2010) Current epidemiological understanding of citrus huanglongbing. *Annu. Rev. Phytopathol.* **48**, 119–139.

Gottwald, T.R. and Graham, J.H. (1992) A device for precise and nondisruptive stomatal inoculation of leaf tissue with bacterial pathogens. *Phytopathology*, **82**, 930–935.

Gottwald, T.R and Irey, M. (2007) Post-hurricane analysis of citrus canker II: predictive model estimation of disease spread and area potentially impacted by various eradication protocols following catastrophic weather events. *Plant Health Progr.* **1**, http://www.apsnet.org/publications/apsnetfeatures/Pages/CitrusCankerII.aspx. doi:10.1094/PHP-2007-0405-1001-RS.

Gottwald, T.R. and Timmer, L.W. (1995) The efficacy of windbreaks in reducing the spread of citrus canker caused by *Xanthomonas campestris* pv *citri*. *Trop. Agric.* **72**, 194–201.

Gottwald, T.R., Alvarez, A.M., Hartung, J.S. and Benedict, A.A. (1991). Diversity of *Xanthomonas campestris* pv *citrumelo* strains associated with epidemics of citrus bacterial spot in Florida citrus nurseries – correlation of detached leaf, monoclonal-antibody, and restriction-fragment-length-polymorphism assays. *Phytopathology*, **81**, 749–753.

Gottwald, T.R., Reynolds, K.M., Campbell, C.L. and Timmer, L.W. (1992) Spatial and spatiotemporal autocorrelation analysis of citrus canker epidemics in citrus nurseries and groves in Argentina. *Phytopathology*, **82**, 843–851.

Gottwald, T.R., Graham, J.H. and Schubert, T.S. (2002) Citrus canker. The pathogen and its impact. *Plant Health Progr.* doi:10.1094/PHP-2002-0812-01-R.

Gottwald, T.R., Bassanezi, R.B., Amorim, L. and Bergamin-Filho, A. (2007) Spatial pattern analysis of citrus canker-infected plantings in Sao Paulo, Brazil, and augmentation of infection elicited by the Asian leafminer. *Phytopathology*, **97**, 674–683.

Graham, J. and Myers, M. (2009) Soil drenches of imidacloprid, thiamethoxam and acibenzolar-S-methyl for induction of SAR to control citrus canker in young citrus trees. *Phytopathology*, **99**, S46–S46.

Graham, J., Dewdney, M. and Myers, M. (2010) Streptomycin and copper formulations for control of citrus canker on grapefruit. *Proc. Florida State Hortic. Soc.* **123**, 92–99.

Graham, J., Dewdney, M. and Yonce, H. (2011) Comparison of copper formulations for control of citrus canker on 'Hamlin' orange. *Proc. Florida State Hortic. Soc.* **124**, 79–84.

Graham, J.H. and Leite, R.P. (2004) Lack of control of citrus canker by induced systemic resistance compounds. *Plant Dis.* **88**, 745–750.

Graham, J.H. and Myers, M.E. (2013) Integration of soil applied neonicotinoid insecticides and acibenzolar-S-methyl for systemic acquired resistance (SAR) control of citrus canker on young citrus trees. *Crop Prot.* **54**, 239–243.

Graham, J.H., Timmer, L.W. and Fardelmann, D. (1986) Toxicity of fungicidal copper in soil to citrus seedlings and vesicular–arbuscular mycorrhizal fungi. *Phytopathology*, **76**, 66–70.

Graham, J.H., Gottwald, T.R., Civerolo, E.L. and Mcguire, R.G. (1989) Population-dynamics and survival of *Xanthomonas campestris* in soil in citrus nurseries in Maryland and Argentina. *Plant Dis.* **73**, 423–427.

Graham, J.H., Gottwald, T.R., Riley, T.D. and Achor, D. (1992) Penetration through leaf stomata and growth of strains of *Xanthomonas campestris* in citrus cultivars varying in susceptibility to bacterial diseases. *Phytopathology*, **82**, 1319–1325.

Graham, J.H., Gottwald, T.R., Cubero, J. and Achor, D.S. (2004) *Xanthomonas axonopodis* pv. *citri*: factors affecting successful eradication of citrus canker. *Mol. Plant Pathol.* **5**, 1–15.

Graham, J.H., Leite, R.P. and Yonce, H.D. (2007) Streptomycin controls citrus canker in Brazil and Florida and reduces risk of copper phytotoxicity on grapefruit. *Phytopathology*, **97**, S42.

Graham, J.H., Brooks, C. and Yonce, H.D. (2016a) Importance of early season copper sprays for protection of Hamlin orange fruit against citrus canker infection and premature fruit drop. *Proc. Florida State Hortic. Soc.* **129**, 74–78.

Graham, J.H., Johnson, E.G., Myers, M.E., Young, M., Rajasekaran, P., Das, S. and Santra, S. (2016b) Potential of nano-formulated zinc oxide for control of citrus canker on grapefruit trees. *Plant Dis.* **100**, 2442–2447.

Guo, Y.P., Sagaram, U.S., Kim, J.S. and Wang, N. (2010) Requirement of the galU gene for polysaccharide production by and pathogenicity and growth *in planta* of *Xanthomonas citri* subsp *citri*. *Appl. Environ. Microbiol.* **76**, 2234–2242.

Guo, Y.P., Figueiredo, F., Jones, J. and Wang, N. (2011) HrpG and hrpX play global roles in coordinating different virulence traits of *Xanthomonas axonopodis* pv. *citri*. *Mol. Plant–Microbe Interact.* **24**, 649–661.

Hacker, J. and Kaper, J.B. (2000) Pathogenicity islands and the evolution of microbes. *Annu. Rev. Microbiol.* **54**, 641–679.

Hall, D.G., Gottwald, T.R. and Bock, C.H. (2010) Exacerbation of citrus canker by citrus leafminer *Phyllocnistis citrella* in Florida. *Fla. Entomol.* **93**, 558–566.

Hartung, J.S. (1992) Plasmid-based hybridization probes for detection and identification of *Xanthomonas campestris* pv *citri*. *Plant Dis.* **76**, 889–893.

Hasse, C.H. (1915) *Pseudomonas citri*, the cause of citrus canker – a preliminary report. *J. Agric. Res.* **4**, 97–100.

He, S.Y. (1998) Type III protein secretion systems in plant and animal pathogenic bacteria. *Annu. Rev. Phytopathol.* **36**, 363–392.

Hoy, M.A. and Jessey, C. (2004) *Ageniaspis citricola* (Hymenoptera: *Encyrtidae*) established in Bermuda. *Fla. Entomol.* **87**, 229–230.

Hsiao, Y.M., Liu, Y.F., Lee, P.Y., Hsu, P.C., Tseng, S.Y. and Pan, Y.C. (2011) Functional characterization of copA gene encoding multicopper oxidase in *Xanthomonas campestris* pv. *campestris*. *J. Agric. Food Chem.* **59**, 9290–9302.

Hu, Y., Zhang, J., Jia, H., White, F., Wang, N., Yang, B. and Jones, J.B. (2013) Diverse TAL effectors converge on a single host susceptibility gene in citrus canker. *Phytopathology*, **103**, 62.

Hu, Y., Zhang, J., Jia, H., Sosso, D., Li, T., Frommer, W.B., Yang, B., White, F.F., Wang, N. and Jones, J.B. (2014) Lateral organ boundaries 1 is a disease susceptibility gene for citrus bacterial canker disease. *Proc. Natl. Acad. Sci. USA*, **111**, E521–E529.

Jaciani, F.J., Ferro, J.A., Ferro, M.I.T., Verniere, C., Pruvost, O. and Belasque, J. (2012) Genetic diversity of a Brazilian strain collection of *Xanthomonas citri* subsp *citri* based on the type III effector protein genes. *Plant Dis.* **96**, 193–203.

Jalan, N., Kumar, D., Andrade, M.O., Yu, F., Jones, J.B., Graham, J.H., White, F.F., Setubal, J.C. and Wang, N. (2013a) Comparative genomic and transcriptome analyses of pathotypes of *Xanthomonas citri* subsp. *citri* provide insights into mechanisms of bacterial virulence and host range. *BMC Genomics*, **14**, 551.

Jalan, N., Kumar, D., Yu, F., Jones, J.B., Graham, J.H. and Wang, N. (2013b) Complete genome sequence of *Xanthomonas citri* subsp. *citri* Strain aw12879, a restricted-host-range citrus canker-causing bacterium. *Genome Announc.* **1**(3), e00235–13. doi:10.1128/genomeA.00235-13.

Jehle, R.A. (1916) Characteristics of citrus canker and of the causal organism. *Q. Bull.* **1**, 2–12.

Jesus, W.C., Belasque, J., Amorim, L., Christiano, R.S.C., Parra, J.R.P. and Bergamin Filho, A. (2006) Injuries caused by citrus leafminer (*Phyllocnistis citrella*) exacerbate citrus canker (*Xanthomonas axonopodis* pv. *citri*) infections. *Fitopatol. Bras.* **31**, 277–283.

Jia, H., Zhang, Y., Orbović, V., Xu, J., White, F.F., Jones, J.B. and Wang, N. (2017) Genome editing of the disease susceptibility gene *CsLob1* in citrus confers resistance to citrus canker. *Plant Biotechnol. J.* **15**, 817–823.

Jia, H.G., Orbovic, V., Jones, J.B. and Wang, N. (2016) Modification of the PthA4 effector binding elements in Type I *CsLOB1* promoter using Cas9/sgRNA to produce transgenic Duncan grapefruit alleviating XccΔpthA4:dCsLOB1.3 infection. *Plant Biotechnol. J.* **14**, 1291–1301.

Johnson, S.J. and Henne, D.C. (2003) Biological control of the citrus leafminer with *Ageniaspis citricola* (Hymenoptera: *Encyrtidae*) in Lousiana. *Proc. Florida State Hortic. Soc.* **116**, 224–226.

Kay, S., Hahn, S., Marois, E., Hause, G. and Bonas, U. (2007) A bacterial effector acts as a plant transcription factor and induces a cell size regulator. *Science*, **318**, 648–651.

Khalaf, A., Moore, G.A., Jones, J.B. and Gmitter, F.G. (2007) New insights into the resistance of Nagami kumquat to canker disease. *Physiol. Mol. Plant.* **71**, 240–250.

Lamed, R. and Bayer, E.A. (1986) Contact and cellulolysis in *Clostridium thermocellum* via extensile surface organelles. *Experientia*, **42**, 72–73.

Lee, H.A. (1921) The increase in resistance to citrus canker with the advance in maturity of citrus trees. *Phytopathology*, **11**, 70–73.

Leite, R., Egel, D. and Stall, R. (1994) Genetic analysis of hrp-related DNA sequences of *Xanthomonas campestris* strains causing diseases of citrus. *Appl. Environ. Microbiol.* **60**, 1078–1086.

Leite, R.P. and Mohan, S.K. (1990) Integrated management of the citrus bacterial canker disease caused by *Xanthomonas campestris* pv *citri* in the state of Parana, Brazil. *Crop Prot.* **9**, 3–7.

Li, J.Y. and Wang, N. (2014) Foliar application of biofilm formation-inhibiting compounds enhances control of citrus canker caused by *Xanthomonas citri* subsp *citri*. *Phytopathology*, **104**, 134–142.

Lin, H., Hsu, S., Hwang, A. and Tzeng, K. (2005) Phenotypic and genetic characterization of novel strains of *Xanthomonas axonopodis* pv. *citri* which induce atypical symptoms on citrus leaves in Taiwan. *Plant Pathol. Bull.* **14**, 227–238.

Lin, H.-C., Chu, M.-K., Lin, Y.-C., Deng, W.-L., Chang, H., Hsu, S.-T. and Tzeng, K.-C. (2011) A single amino acid substitution in pthA of *Xanthomonas axonopodis* pv. *citri* altering canker formation on grapefruit leaves. *Eur. J. Plant Pathol.* **130**, 143–154.

Lin, H.-C., Chang, Y.-A. and Chang, H. (2013) A pthA homolog from a variant of *Xanthomonas axonopodis* pv. *citri* enhances virulence without inducing canker symptom. *Eur. J. Plant Pathol.* **137**, 677–688.

Louws, F.J., Rademaker, J.L.W. and de Bruijn, F.J. (1999) The three Ds of PCR-based genomic analysis of phytobacteria: diversity, detection, and disease diagnosis. *Annu. Rev. Phytopathol.* **37**, 81–125.

Louws, F.J., Wilson, M., Campbell, H.L., Cuppels, D.A., Jones, J.B., Shoemaker, P.B., Sahin, F. and Miller, S.A. (2001) Field control of bacterial spot and bacterial speck of tomato using a plant activator. *Plant Dis.* **85**, 481–488.

Ludidi, N., Morse, M., Sayed, M., Wherrett, T., Shabala, S. and Gehring, C. (2004) A recombinant plant natriuretic peptide causes rapid and spatially differentiated K^+, Na^+ and H^+ flux changes in *Arabidopsis thaliana* roots. *Plant Cell Physiol.* **45**, 1093–1098.

Mafi, S. and Ohbayashi, N. (2010) Biology of *Chrysocharis pentheus*, an endoparasitoid wasp of the citrus leafminer *Phyllocnistis citrella* Stainton. *J. Agric. Sci. Technol.* **12**, 145–154.

Malamud, F., Homem, R.A., Conforte, V.P., Yaryura, P.M., Castagnaro, A.P., Marano, M.R., do Amaral, A.M. and Vojnov, A.A. (2013) Identification and characterization of biofilm formation-defective mutants of *Xanthomonas citri* subsp. *citri*. *Microbiology*, **159**, 1911–1919.

Maryani, M.M., Bradley, G., Cahill, D.M. and Gehring, C.A. (2001) Natriuretic peptides and immunoreactants modify osmoticum-dependent volume changes in *Solanum tuberosum* L. mesophyll cell protoplasts. *Plant Sci.* **161**, 443–452.

Mavrodieva, V., Levy, L. and Gabriel, D.W. (2004) Improved sampling methods for real-time polymerase chain reaction diagnosis of citrus canker from field samples. *Phytopathology*, **94**, 61–68.

Mellano, M.A. and Cooksey, D.A. (1988) Nucleotide-sequence and organization of copper resistance genes from *Pseudomonas syringae* pv *tomato*. *J. Bacteriol.* **170**, 2879–2883.

Mendes, B.M.J., Cardoso, S.C., Boscariol-Camargo, R.L., Cruz, R.B., Mourão Filho, F.A.A. and Bergamin Filho, A. (2010) Reduction in susceptibility to *Xanthomonas axonopodis* pv. *citri* in transgenic *Citrus sinensis* expressing the rice xa21 gene. *Plant Pathol.* **59**, 68–75.

Menkissoglu, O. and Lindow, S.E. (1991) Chemical forms of copper on leaves in relation to the bactericidal activity of cupric hydroxide deposits on plants. *Phytopathology*, **81**, 1263–1270.

Miller, J.R., Gut, L.J., de Lame, F.M. and Stelinski, L.L. (2006) Differentiation of competitive vs. non-competitive mechanisms mediating disruption of moth sexual communication by point sources of sex pheromone (Part I): theory. *J. Chem. Ecol.* **32**, 2089–2114.

Moreira, L.M., Almeida, N.F., Potnis, N., Digiampietri, L.A., Adi, S.S., Bortolossi, J.C., da Silva, A.C., da Silva, A.M., de Moraes, F.E., de Oliveira, J.C., de Souza, R.F., Fancincani, A.P., Ferraz, A.L., Ferro, M.I., Furlan, L.R., Gimenez, D.F., Jones, J.B., Kitajima, E.W., Laia, M.L., Leite, R.P., Nishiyama, M.Y., Rodrigues Neto, J., Nociti, L.A., Norman, D.J., Ostroski, E.H., Pereira, H.A., Staskawicz, B.J., Tezza, R.I., Ferro, J.A., Vinatzer, B.A. and Setubal, J.C. (2010) Novel insights into the genomic basis of citrus canker based on the genome sequences of two strains of *Xanthomonas fuscans* subsp. *aurantifolii*. *BMC Genomics*, **11**, 238.

Moschini, R.C., Canteros, B.I., Martinez, M.I. and De Ruyver, R. (2014) Quantification of the environmental effect on citrus canker intensity at increasing distances from a natural windbreak in northeastern Argentina. *Australas Plant Pathol.* **43**, 653–662.

Mysore, K.S. and Ryu, C.M. (2004) Nonhost resistance: how much do we know? *Trends Plant Sci.* **9**, 97–104.

Namekata, T. and Oliveira, A.D. (1972) Comparative serological studies between *Xanthomonas citri* and a bacterium causing canker on Mexican lime. In: *Proceedings of the Third International Conference on Plant Pathogenic Bacteria* (Maas Geesteranus, H.P. eds), pp. 151–2. Wageningen, the Netherlands: Centre of the Agricultural Publication and Documentation.

Ngoc, L.B.T., Vernière, C., Boyer, C., Vital, K., Pruvost, O., Le Mai, N. and Le Thi Thu, H. (2009) Pathotype identification of *Xanthomonas citri* pv. *citri* strains causing citrus canker in Vietnam. *Plant Dis.* **93**, 671.

Ngoc, L.B.T., Verniere, C., Jouen, E., Ah-You, N., Lefeuvre, P., Chiroleu, F., Gagnevin, L. and Pruvost, O. (2010) Amplified fragment length polymorphism and multilocus sequence analysis-based genotypic relatedness among pathogenic variants of *Xanthomonas citri* pv. *citri* and *Xanthomonas campestris* pv. *bilvae*. *Int. J. Syst. Evol. Microbiol.* **60**, 515–525.

Obradovic, A., Mavridis, A., Rudolph, K., Janse, J.D., Arsenijevic, M., Jones, J.B., Minsavage, G.V. and Wang, J.-F. (2004) Characterization and PCR-based typing of *Xanthomonas campestris* pv. *vesicatoria* from peppers and tomatoes in Serbia. *Eur. J. Plant Pathol.* **110**, 285–292.

de Oliveira, M.L.P., Silva, C.C.D., Abe, V.Y., Costa, M.G.C., Cernadas, R.A. and Benedetti, C.E. (2013) Increased resistance against citrus canker mediated by a citrus mitogen-activated protein kinase. *Mol. Plant–Microbe Interact.* **26**, 1190–1199.

O'Toole, G., Kaplan, H.B. and Kolter, R. (2000) Biofilm formation as microbial development. *Annu. Rev. Microbiol.* **54**, 49–79.

Park, D.S., Wook Hyun, J., Jin Park, Y., Sun Kim, J., Wan Kang, H., Ho Hahn, J. and Joo Go, S. (2006) Sensitive and specific detection of *Xanthomonas axonopodis* pv. *citri* by PCR using pathovar specific primers based on hrpW gene sequences. *Microbiol. Res.* **161**, 145–149.

Pena, J.E., Hunsberger, A. and Schaffer, B. (2000) Citrus leafminer (Lepidoptera: *Gracillariidae*) density: effect on yield of 'Tahiti' lime. *J. Econ. Entomol.* **93**, 374–379.

Pereira, A.L.A., Carazzolle, M.F., Abe, V.Y., de Oliveira, M.L.P., Domingues, M.N., Silva, J.C., Cernadas, R.A. and Benedetti, C.E. (2014) Identification of putative TAL effector targets of the citrus canker pathogens shows functional convergence underlying disease development and defense response. *BMC Genomics*, **15**, 157.

Petrocelli, S., Tondo, M.L., Daurelio, L.D. and Orellano, E.G. (2012) Modifications of *Xanthomonas axonopodis* pv. *citri* lipopolysaccharide affect the basal response and the virulence process during citrus canker. *PLoS One*, **7**, e40051

Pharmawati, M., Maryani, M.M., Nikolakopoulos, T., Gehring, C.A. and Irving, H.R. (2001) Cyclic GMP modulates stomatal opening induced by natriuretic peptides and immunoreactive analogues. *Plant Physiol. Biochem.* **39**, 385–394.

Popham, P.L., Pike, S.M., Novacky, A. and Pallardy, S.G. (1993) Water relation alterations observed during hypersensitive reaction induced by bacteria. *Plant Physiol.* **103**, 1243–1247.

Powell, C.A., Burton, M.S., Pelosi, R., Ritenour, M.A. and Bullock, R.C. (2007) Seasonal abundance and insecticidal control of citrus leafminer in a citrus orchard. *Hortscience*, **42**, 1636–1638.

Pruvost, O., Hartung, J.S., Civerolo, E.L., Dubois, C. and Perrier, X. (1992) Plasmid DNA fingerprints distinguish pathotypes of *Xanthomonas campestris* pv *citri*, the causal agent of citrus bacterial canker disease. *Phytopathology*, **82**, 485–490.

Pruvost, O., Boher, B., Brocherieux, C., Nicole, M. and Chiroleu, F. (2002) Survival of *Xanthomonas axonopodis* pv. *citri* in leaf lesions under tropical environmental conditions and simulated splash dispersal of inoculum. *Phytopathology*, **92**, 336–346.

Pruvost, O., et al., (2015) Genetic structure analysis of strains causing citrus canker in Iran reveals the presence of two different lineages of *Xanthomonas citri* pv *citri* pathotype A*. *Plant Pathology*, **64**(4), 776–784, ISSN 0032-0862, doi: 10.1111/ppa.12324.

Rigano, L.A., Siciliano, F., Enrique, R., Sendín, L., Filippone, P., Torres, P.S., Qüesta, J., Dow, J.M., Castagnaro, A.P., Vojnov, A.A. and Marano, M.R. (2007) Biofilm formation, epiphytic fitness, and canker development in *Xanthomonas axonopodis* pv *citri*. *Mol. Plant–Microbe Interact.* **20**, 1222–1230.

Rigano, L.A., Marano, M.R., Castagnaro, A.P., Do Amaral, A.M. and Vojnov, A.A. (2010) Rapid and sensitive detection of citrus bacterial canker by loop-mediated isothermal amplification combined with simple visual evaluation methods. *BMC Microbiol.* **10**, 176.

Rogers, M.E. (2012) Protection of young citrus trees from Asian citrus psyllid and HLB. *Physiol. Plant Pathol.* **93**, 10–15.

Rogers, M.E., Stansly, P.A. and Stelinski, L.L. (2015) Asian citrus psyllid and citrus leafminer. In: *Florida Citrus Pest Management Guide* (Roger, M.E. and Dewdney, M.M., eds), pp. 89–91. Gainesville, FL: University of Florida, IFAS.

Rossier, O., Van den Ackerveken, G. and Bonas, U. (2000) HrpB2 and hrpF from *Xanthomonas* are type III-secreted proteins and essential for pathogenicity and recognition by the host plant. *Mol. Microbiol.* **38**, 828–838.

Rosetti, V. (1977) Citrus canker in Latin America: a review. In: *International Citrus Congress 2nd: 1977: Orlando, Florida*. Orlando, Florida: International Society of Citriculture, p. 3.

Rybak, M., Minsavage, G.V., Stall, R.E. and Jones, J.B. (2009) Identification of *Xanthomonas citri* ssp *citri* host specificity genes in a heterologous expression host. *Mol. Plant Pathol.* **10**, 249–262.

Scapin, M.D., Behlau, F., Scandelai, L.H.M., Fernandes, R.S., Silva, G.J. and Ramos, H.H. (2015) Tree-row-volume-based sprays of copper bactericide for control of citrus canker. *Crop Prot.* **77**, 119–126.

Schaad, N.W., Jones, J.B. and Chun, W. (2001) *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. St. Paul, MN: American Phytopathological Society.

Schaad, N.W., Postnikova, E., Lacy, G., Sechler, A., Agarkova, I., Stromberg, P.E., Stromberg, V.K. and Vidaver, A.K. (2005) Reclassification of *Xanthomonas campestris* pv. *citri* (ex Hasse 1915) Dye 1978 forms A, B/C/D, and E as *X. smithii* subsp. *citri* (ex Hasse) sp. nov. nom. rev. comb. nov., *X. fuscans* subsp. *aurantifolii* (ex Gabriel 1989) sp. nov. nom. rev. comb. nov., and *X. alfalfae* subsp. *citrumelo* (ex Riker and Jones) Gabriel *et al*., 1989 sp. nov. nom. rev. comb. nov.; *X. campestris* pv. *malvacearum* (ex Smith 1901) Dye 1978 as *X. smithii* subsp. *smithii* nov. comb. nov. nom. nov.; *X. campestris* pv. *alfalfae* (ex Riker and Jones, 1935) Dye 1978 as *X. alfalfae* subsp. *alfalfae* (ex Riker *et al*., 1935) sp. nov. nom. rev.; and ''var. *fuscans*'' of *X. campestris* pv. *phaseoli* (ex Smith, 1987) Dye 1978 as *X. fuscans* subsp. *fuscans* sp. nov. *Syst. Appl. Microbiol.* **28**, 494–518.

Schaad, N.W., Postnikova, E., Lacy, G., Sechler, A., Agarkova, I., Stromberg, P.E., Stromberg, V.K. and Vidaver, A.K. (2006) Emended classification of Xanthomonad pathogens on citrus. *Syst. Appl. Microbiol.* **29**, 690–695.

Schubert, T.S., Rizvi, S.A., Sun, X., Gottwald, T.R., Graham, J.H. and Dixon, W.N. (2001) Meeting the challenge of eradicating citrus canker in Florida – again. *Am. Phytopathol. Soc.* **85**, 340–356.

Sena-Vélez, M., Redondo, C., Gell, I., Ferragud, E., Johnson, E., Graham, J.H. and Cubero, J. (2015) Biofilm formation and motility of *Xanthomonas* strains with different citrus host range. *Plant Pathol.* **64**, 767–775.

Sena-Vélez, M., Redondo, C., Graham, J.H. and Cubero, J. (2016) Presence of extracellular DNA during biofilm formation by *Xanthomonas citri* subsp *citri* strains with different host range. *PLoS One*, **11**, e0156695.

Sendin, L.N., Filippone, M.P., Orce, I.G., Rigano, L., Enrique, R., Peña, L., Vojnov, A.A., Marano, M.R. and Castagnaro, A.P. (2012) Transient expression of pepper bs2 gene in *Citrus limon* as an approach to evaluate its utility for management of citrus canker disease. *Plant Pathol.* **61**, 648–657.

Shantharaj, D., Minsavage, J., Stall, R., Lahaye, T., Strauss, A., Hu, Y. and Horvath, D.(2013) Deciphering specificities of TAL effectors in *Xanthomonas citri* and prospects in citrus. *Phytopathology*, **103**, S2.

Shi, Q.C., Febres, V.J., Jones, J.B. and Moore, G.A. (2015) Responsiveness of different citrus genotypes to the *Xanthomonas citri* ssp *citri*-derived pathogen-associated molecular pattern (PAMP) flg22 correlates with resistance to citrus canker. *Mol. Plant Pathol.* **16**, 507–520.

Shiotani, H., Fujikawa, T., Ishihara, H., Tsuyumu, S. and Ozaki, K. (2007) A pthA homolog from *Xanthomonas axonopodis* pv. *citri* responsible for host-specific suppression of virulence. *J. Bacteriol.* **189**, 3271–3279.

Siciliano, F., Torres, P., Sendin, L., Bermejo, C., Filippone, P., Vellice, G., Ramallo, J., Castagnaro, A., Vojnov, A. and Marano, M.R. (2006) Analysis of the molecular basis of *Xanthomonas axonopodis* pv. *citri* pathogenesis in *Citrus limon*. *Electron J. Biotechnol.* **9**, 199–204.

da Silva, A.C.R., Ferro, J.A., Reinach, F.C., Farah, C.S., Furlan, L.R., Quaggio, R.B., Monteiro-Vitorello, C.B., Van Sluys, M.A., Almeida, N.F., Alves, L.M.C., do Amaral, A.M., Bertolini, M.C., Camargo, L.E.A., Camarotte, G., Cannavan, F., Cardozo, J., Chambergo, F., Ciapina, L.P., Cicarelli, R.M.B., Coutinho, L.L., Cursino-Santos, J.R., El-Dorry, H., Faria, J.B., Ferreira, A.J.S., Ferreira, R.C.C., Ferro, M.I.T., Formighieri, E.F., Franco, M.C., Greggio, C.C., Gruber, A., Katsuyama, A.M., Kishi, L.T., Leite, R.P., Lemos, E.G.M., Lemos, M.V.F., Locali, E.C., Machado, M.A., Madeira, A.M.B.N., Martinez-Rossi, N.M., Martins, E.C., Meidanis, J., Menck, C.F.M., Miyaki, C.Y., Moon, D.H., Moreira, L.M., Novo, M.T.M., Okura, V.K., Oliveira, M.C., Oliveira, V.R., Pereira, H.A., Rossi, A., Sena, J.A.D., Silva, C., de Souza, R.F., Spinola, L.A.F., Takita, M.A., Tamura, R.E., Teixeira, E.C., Tezza, R.I.D., Trindade dos Santos, M., Truffi, D., Tsai, S.M., White, F.F., Setubal, J.C. and Kitajima, J.P. (2002) Comparison of the genomes of two *Xanthomonas* pathogens with differing host specificities. *Nature*, **417**, 459–463.

Slater, H., Alvarez-Morales, A., Barber, C.E., Daniels, M.J. and Dow, J.M. (2000) A two-component system involving an HD-GYP domain protein links cell–cell signalling to pathogenicity gene expression in *Xanthomonas campestris*. *Mol. Microbiol.* **38**, 986–1003.

Society of American Bacteriologists., **Bergey, D.H.** and Katherine Golden Bitting Collection on Gastronomy (Library of Congress). (1923) *Bergey's Manual of Determinative Bacteriology; a Key for the Identification of Organisms of the Class Schizomycetes*. Baltimore, MA: Williams & Wilkins Company.

Soprano, A.S., Abe, V.Y., Smetana, J.H. and Benedetti, C.E. (2013) Citrus maf1, a repressor of RNA pol III, binds the *Xanthomonas citri* canker elicitor pthA4 and suppresses citrus canker development. *Plant Physiol.* **163**, 232–242.

Stall, R., Miller, J., Marco, G. and Canteros de Echenique, B. (1982) Timing of sprays to control cancrosis of grapefruit in Argentina. In: *Proceedings of the International Society of Citriculture/[International Citrus Congress, November 9–12, 1981, Tokyo, Japan* (Matsumoto, K., ed.), pp. 414–417. Shimizu, Japan: International Society of Citriculture.

Stein, B., Ramallo, J., Foguet, L. and Graham, J.H. (2007) Citrus leafminer control and copper sprays for management of citrus canker on lemon in Tucuman, Argentina. *Proc. Florida State Hortic. Soc.* **120**, 127–131.

Stelinski, L.L. and Czokajlo, D. (2010) Suppression of citrus leafminer, *Phyllocnistis citrella*, with an attract-and-kill formulation. *Entomol. Exp. Appl.* **134**, 69–77.

Stelinski, L.L., Miller, J.R. and Rogers, M.E. (2008) Mating disruption of citrus leafminer mediated by a noncompetitive mechanism at a remarkably low pheromone release rate. *J. Chem. Ecol.* **34**, 1107–1113.

Stelinski, L.L., Lapointe, S.L. and Meyer, W.L. (2010) Season-long mating disruption of citrus leafminer, *Phyllocnistis citrella* Stainton, with an emulsified wax formulation of pheromone. *J. Appl. Entomol.* **134**, 512–520.

Stevens, H.E. (1914) Citrus canker. A preliminary bulletin. *Florida Agric. Expt. Sta. Bull.* **122**, 113–118.

Stoodley, P., Sauer, K., Davies, D.G. and Costerton, J.W. (2002) Biofilms as complex differentiated communities. *Annu. Rev. Microbiol.* **56**, 187–209.

Sun, X., Stall, R.E., Jones, J.B., Cubero, J., Gottwald, T.R., Graham, J.H., Dixon, W.N., Schubert, T.S., Chaloux, P.H., Stromberg, V.K., Lacy, G.H. and Sutton, B.D. (2004) Detection and characterization of a new strain of citrus canker bacteria from key Mexican lime and alemow in South Florida. *Plant Dis.* **88**, 1179–1188.

Sundin, G.W., Jones, A.L. and Fulbright, D.W. (1989) Copper resistance in *Pseudomonas syringae* pv *syringae* from cherry orchards and its associated transfer *in vitro* and *in planta* with a plasmid. *Phytopathology*, **79**, 861–865.

Swarup, S., Defeyter, R., Brlansky, R.H. and Gabriel, D.W. (1991) A pathogenicity locus from *Xanthomonas citri* enables strains from several pathovars of *Xanthomonas campestris* to elicit cankerlike lesions on citrus. *Phytopathology*, **81**, 802–809.

Swarup, S., Yang, Y.N., Kingsley, M.T. and Gabriel, D.W. (1992) An *Xanthomonas citri* pathogenicity gene, ptha, pleiotropically encodes gratuitous avirulence on nonhosts. *Mol. Plant–Microbe Interact.* **5**, 204–213.

Tally, A., Oostendorp, M., Lawton, K., Staub, T. and Bassi, B. (1999) Commercial development of elicitors of induced resistance to pathogens. In: *Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture* (Agrawal, A.A., Tuzun, S. and Bent, E., eds), pp. 357–369. St. Paul, MN: American Phytopathological Society Press.

Tampakaki, A.P., Skandalis, N., Gazi, A.D., Bastaki, M.N., Sarris, P.F., Charova, S.N., Kokkinidis, M. and Panopoulos, N.J. (2010) Playing the "harp": evolution of our understanding of hrp/hrc genes 1. *Annu. Rev. Phytopathol.* **48**, 347–370.

Tang, J.L., Liu, Y.N., Barber, C.E., Dow, J.M., Wootton, J.C. and Daniels, M.J. (1991) Genetic and molecular analysis of a cluster of rpf genes involved in positive regulation of synthesis of extracellular enzymes and polysaccharide in *Xanthomonas campestris* pathovar *campestris*. *Mol. Gen. Genet.* **226**, 409–417.

Teixeira, E.C., de Oliveira, J.C.F., Novo, M.T.M. and Bertolini, M.C. (2008) The copper resistance operon copAB from *Xanthomonas axonopodis* pathovar *citri*: gene inactivation results in copper sensitivity. *Microbiology*, **154**, 402–412.

Timmer, L.W., Gottwald, T.R. and Zitko, S.E. (1991) Bacterial exudation from lesions of Asiatic citrus canker and citrus bacterial spot. *Plant Dis.* **75**, 192–195.

Van Sluys, M.A., Monteiro-Vitorello, C.B., Camargo, L.E.A., Menck, C.F.M., da Silva, A.C.R., Ferro, J.A., Oliveira, M.C., Setubal, J.C., Kitajima, J.P. and Simpson, A.J. (2002) Comparative genomic analysis of plant-associated bacteria. *Annu. Rev. Phytopathol.* **40**, 169–189.

Vauterin, L., Swings, J. and Kersters, K. (1991a) Grouping of *Xanthomonas campestris* pathovars by SDS-PAGE of proteins. *J. Gen. Microbiol.* **137**, 1677–1687.

Vauterin, L., Yang, P., Hoste, B., Vancanneyt, M., Civerolo, E.L. and Swings, J and Kersters, K. (1991b) Differentiation of *Xanthomonas campestris* pv *citri* strains by sodium dodecyl sulfate-polyacrylamide gel-electrophoresis of proteins, fatty-acid analysis, and DNA–DNA hybridization. *Int. J. Syst. Bacteriol.* **41**, 535–542.

Vauterin, L., Hoste, B., Kersters, K. and Swings, J. (1995) Reclassification of *Xanthomonas*. *Int. J. Syst. Bacteriol.* **45**, 472–489.

Vauterin, L., Yang, P., Alvarez, A., Takikawa, Y., Roth, D.A., Vidaver, A.K., Stall, R.E., Kersters, K. and Swings, J. (1996a) Identification of non-pathogenic Xanthomonas strains associated with plants. *Syst. Appl. Microbiol.* **19**, 96–105.

Vauterin, L., Yang, P. and Swings, J. (1996b) Utilization of fatty acid methyl esters for the differentiation of new Xanthomonas species. *Int. J. Syst. Bacteriol.* **46**, 298–304.

Verniere, C., Devaux, M., Pruvost, O., Couteau, A. and Luisetti, J. (1991) Studies on the biochemical and physiological variations among strains of *Xanthomonas campestris* pv. *citri*, the causal agent of citrus bacterial canker disease. *Fruits*, **46**, 162–170.

Verniere, C., Hartung, J.S., Pruvost, O.P., Civerolo, E.L., Alvarez, A.M., Maestri, P. and Luisetti, J. (1998) Characterization of phenotypically distinct strains of *Xanthomonas axonopodis* pv. *citri* from Southwest Asia. *Eur. J. Plant Pathol.* **104**, 477–487.

Verniere, C.J., Gottwald, T.R. and Pruvost, O. (2003) Disease development and symptom expression of *Xanthomonas axonopodis* pv. *citri* in various citrus plant tissues. *Phytopathology*, **93**, 832–843.

Vojnov, A.A., Zorreguieta, A., Dow, J.M., Daniels, M.J. and Dankert, M.A. (1998) Evidence for a role for the gumB and gumC gene products in the formation of xanthan from its pentasaccharide repeating unit by *Xanthomonas campestris*. *Microbiology*, **144**, 1487–1493.

Voloudakis, A.E., Reignier, T.M. and Cooksey, D.A. (2005) Regulation of resistance to copper in *Xanthomonas axonopodis* pv. *vesicatoria*. *Appl. Environ. Microbiol.* **71**, 782–789.

Wang, Y. and Liu, J.H. (2012) Exogenous treatment with salicylic acid attenuates occurrence of citrus canker in susceptible navel orange (*Citrus sinensis* Osbeck). *J. Plant Physiol.* **169**, 1143–1149.

Yamazaki, A., Hirata, H. and Tsuyumu, S. (2008a) HrpG regulates type II secretory proteins in *Xanthomonas axonopodis* pv. *citri*. *J. Gen. Plant Pathol.* **74**, 138–150.

Yamazaki, A., Hirata, H. and Tsuyumu, S. (2008b) Type III regulators hrpG and hrpXct control synthesis of alpha-amylase, which is involved in in planta multiplication of *Xanthomonas axonopodis* pv. *citri*. *J. Gen. Plant Pathol.* **74**, 254–257.

Yan, Q. and Wang, N.A. (2011) The colR/colS two-component system plays multiple roles in the pathogenicity of the citrus canker pathogen *Xanthomonas citri* subsp *citri*. *J. Bacteriol.* **193**, 1590–1599.

Yan, Q. and Wang, N. (2012) High-throughput screening and analysis of genes of *Xanthomonas citri* subsp *citri* involved in citrus canker symptom development. *Mol. Plant–Microbe Interact.* **25**, 69–84.

Yang, Y.N. and Gabriel, D.W. (1995) *Xanthomonas* avirulence/pathogenicity gene family encodes functional-plant nuclear targeting signals. *Mol. Plant–Microbe Interact.* **8**, 627–631.

Ye, G., Hong, N., Zou, L.-F., Zou, H.-S., Zakria, M., Wang, G.-P. and Chen, G.-Y. (2013) TALE-based genetic diversity of Chinese isolates of the citrus canker pathogen *Xanthomonas citri* subsp. *citri*. *Plant Dis.* **97**, 1187–1194.

Young, J.M., Dye, D.W., Bradbury, J.F., Panagopoulos, C.G. and Robbs, C.F. (1978) Proposed nomenclature and classification for plant pathogenic bacteria. *N. Z. J. Agric. Res.* **21**, 153–177.

Young, J.M., Bradbury, J.F., Gardan, L., Gvozdyak, R.I., Stead, D.E., Takikawa, Y. and Vidaver, A.K. (1991) Comment on the reinstatement of *Xanthomonas citri* (Ex Hasse 1915) Gabriel *et al* 1989 and *X. phaseoli* (Ex Smith 1897) Gabriel *et al* 1989 – indication of the need for minimal standards for the genus *Xanthomonas*. *Int. J. Syst. Bacteriol.* **41**, 172–177.

Yun, M.H., Torres, P.S., El Oirdi, M., Rigano, L.A., Gonzalez-Lamothe, R., Marano, M.R., Castagnaro, A.P., Dankert, M.A., Bouarab, K. and Vojnov, A.A. (2006) Xanthan induces plant susceptibility by suppressing callose deposition. *Plant Physiol.* **141**, 178–187.

Zhang, X.D., Francis, M.I., Dawson, W.O., Graham, J.H., Orbovic, V., Triplett, E.W. and Mou, Z. (2010) Over-expression of the Arabidopsis NPR1 gene in citrus increases resistance to citrus canker. *Eur. J. Plant Pathol.* **128**, 91–100.

Zhang, Y., Jalan, N., Zhou, X., Goss, E., Jones, J.B., Setubal, J.C., Deng, X. and Wang, N. (2015) Positive selection is the main driving force for evolution of citrus canker-causing *Xanthomonas*. *ISME J.* **9**, 2128–2138.

Zhang, Y.X., Xu, S.H., Ding, P.T., Wang, D.M., Cheng, Y.T., He, J., Gao, M., Xu, F., Li, Y., Zhu, Z., Li, X. and Zhang, Y. (2010) Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. *Proc. Natl. Acad. Sci. USA*, **107**, 18 220–18 225.

Zimaro, T., Thomas, L., Marondedze, C., Garavaglia, B.S., Gehring, C., Ottado, J. and Gottig, N. (2013) Insights into *Xanthomonas axonopodis* pv. *citri* biofilm through proteomics. *BMC Microbiol.* **13**, 1–14.

Zimaro, T., Thomas, L., Marondedze, C., Sgro, G.G., Garofalo, C.G., Ficarra, F.A., Gehring, C., Ottado, J. and Gottig, N. (2014) The type III protein secretion system contributes to *Xanthomonas citri* subsp *citri* biofilm formation. *BMC Microbiol.* **14**, 1–15.