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Review Article

The psorosis disease of citrus: a pale light at the end of the tunnel.

P Moreno[1*], J Guerri[1], and ML García[2]

[1] Centro de Protección Vegetal y Biotecnología, Instituto Valenciano de Investigaciones Agrarias, Moncada, 46113-Valencia, Spain; [2] Instituto de Biotecnología y Biología Molecular, Department of Biological Sciences, Facultad de Ciencias Exactas, Universidad Nacional de la Plata (UNLP), CONICET – UNLP, La Plata, Argentina.

*Correspondence to: pedromoreno.ivia@gmail.com

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Abstract

First reported in 1896, psorosis was the first citrus disease proven to be graft transmissible and also the first for which eradication and budwood certification programs were launched to prevent its economic damage. For many years psorosis etiology remained elusive, and only in 1986 was the disease associated with the presence of virus-like particles in infected plants. However, in the last 2 decades a virus with unusual morphology (*Citrus psorosis virus*, CPsV) was characterized and closely associated with psorosis disease as previously defined by field symptoms and by biological indexing in sensitive indicator plants. With a tripartite, negative-sense, RNA genome and a ~48 kDa coat protein, CPsV, the presumed causal agent of psorosis, is the type member of the genus *Ophiovirus*, within the new family *Ophioviridae*. Availability of the complete genomic sequence of 2 CPsV isolates and partial sequences of many others has enabled i) setting up rapid and sensitive RNA-based detection methods, ii) testing different citrus and relatives for resistance to CPsV, iii) identification of the 2 components (psorosis A and psorosis B) traditionally associated with non-scaled and scaled bark inoculum, respectively, from psorosis-infected plants and study their interactions, iv) analysis of genetic variation and evolutionary forces shaping the CPsV populations, v) preliminary studies on the interactions between virus and host factors, and vi) development of transgenic citrus plants expressing variable degrees of resistance to CPsV. In summary, 120 years after the first report on psorosis we start seeing a pale light at the end of the tunnel.

Keywords: psorosis A, psorosis B, *Citrus psorosis virus* (CPsV), *Ophiovirus*, symptoms, detection, characterization, genetic variation, citrus resistance to CPsV

Introduction

Psorosis is a citrus disease that may affect trunk and branches, leaves and fruits, causing growth reduction, thin foliage, low fruit bearing and tree decline. In the field, the most characteristic symptom of affected trees is bark scaling in the trunk and branches with gum production and wood discoloration below the bark lesions (Fig. 1a to 1c). The lesions may be limited to some areas of the stem and main branches (Fig. 1a), a syndrome called psorosis A (PsA), or they may be rampant and affect even thin branches sloughing large strips of bark (Fig. 1b), a more aggressive syndrome known as psorosis B (PsB). Sometimes, young leaves of the spring flush show different chlorotic patterns (flecking, blotching, or ring spots) and some new shoots of the spring flush may show a shock reaction with leaf shedding and shoot necrosis, and in the case of the PsB type, the old leaves often show chlorotic blotches in the upper side with gum-impregnated brownish eruptions in the underside (Fig. 1d and 1f). The fruits of the PsB affected trees may have depressed spots or rings in the rind with discolored tissue Fig. 1e).

In the greenhouse, young seedlings of sensitive indicator species graft-inoculated with psorosis usually display the shock reaction in the first flush (Fig. 2a) and transient chlorotic flecks, blotches or ringspots in young leaves of the following flushes (Fig. 2c to 2e). PsB isolates additionally show chlorotic blotching in old leaves as in the field (Fig. 2f and 2g) and blisters in the stem and twigs (Fig. 2b).

Reported by Swingle and Webber (1896) as a bark scaling disorder of citrus trees, psorosis is the first citrus disease proven to be graft transmissible (Fawcett 1933, 1934). It was also the first citrus disease for which eradication and budwood certification programs were launched to prevent its economic damage (Doidge 1926; Fawcett 1938). However, for many years it was one of the citrus diseases considered of recalcitrant etiology (Derrick and Timmer 2000) and it was not until 1986 that this

disease was associated with the presence in infected plants of virus-like particles and a ~48 kDa protein (Derrick et al. 1988a; da Graça et al. 1991), that later was shown to be the viral coat protein (Barthe et al. 1998; Sánchez de la Torre et al. 1998).

Fig. 1. Field symptoms of psorosis disease. a) Bark scaling and gumming restricted to the stem and main branches of a sweet orange, characteristic of psorosis A (PsA); b) Rampant bark scaling affecting thin branches of a sweet orange, characteristic of PsB; c) Discoloration affecting wood below the bark lesions; d) Yellow blotches in the upper side of some old leaves of a Marsh grapefruit affected by PsB; e) Depressed areas with discolored tissue in the rind of fruits of a PsB-affected sweet orange tree; and f) Gum-impregnated brownish eruptions in the underside of the grapefruit leaves (d) affected by PsB.

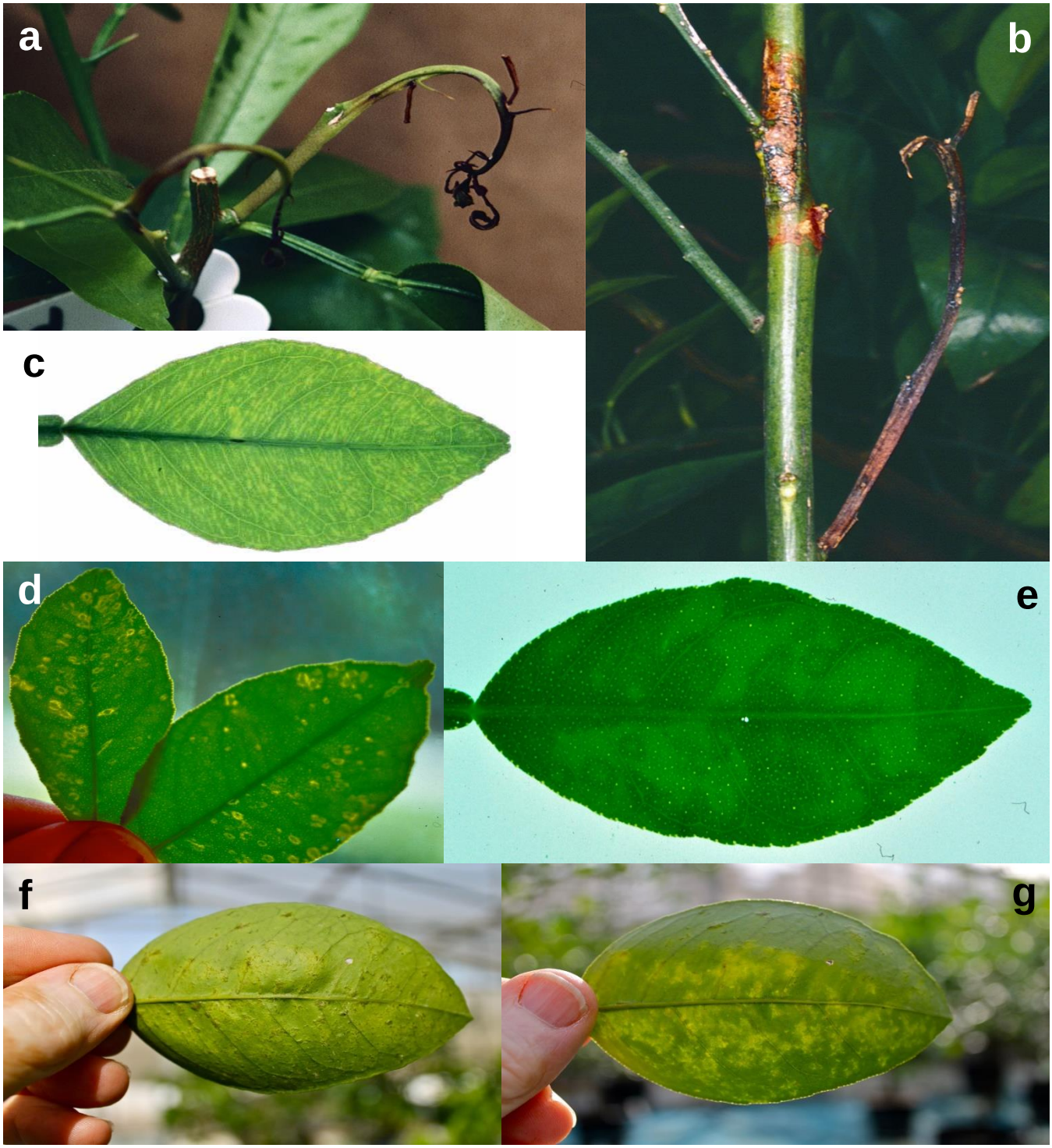

Fig. 2. Psorosis A and B (PsA and PsB) symptoms observed by biological indexing in the greenhouse. a) Shock reaction with leaf shedding and shoot necrosis observed in the first flush of a sweet orange seedling inoculated with psorosis; b) Blisters in a green twig of a sweet orange inoculated with PsB; c-e) Chlorotic flecks (c), ring spots (d) or blotches induced by psorosis in young leaves of the second and successive flushes; f-g) Chlorotic blotches (g) and gum-impregnated pustules in the leaf underside (f) induced by PsB in old leaves of a sweet orange seedling.

In this long period, a lot of confusion was generated in the literature on the symptoms induced by psorosis and other related and non-related diseases and disorders affecting citrus trees that were called 'psorosis group' (Fawcett and Bitancourt 1943; Wallace 1978). This confusion was generated by i) the similarity of symptoms induced in young leaves of indicator plants by several diseases causing different field symptoms, namely psorosis, citrus ringspot, concave gum-blind pocket, impietratura, cristacortis, and infectious-variegation-crinkly leaf (Duran-Vila and Moreno 2000; Timmer et al. 2000), ii) the long period required by psorosis-infected

plants to develop bark scaling, its most characteristic symptom, in comparison with the non-specific young leaf symptoms, which made it difficult to know if candidate field trees showing symptoms other than bark scaling were actually infected with both psorosis and the disease being tested, iii) the use of a cross protection test (see below) for psorosis diagnostics with trees doubly infected with psorosis and a non-psorosis disease, and iv) the erroneous association of psorosis with different types of bark scaling or ringspot symptoms in old leaves or fruits induced in field trees by other biotic or abiotic agents.

Details on the origin and evolution of this controversial 'psorosis group' have been described or reviewed in several papers (Wallace 1968, 1978; Broadbent 1972; Broadbent and Fraser 1980; Timmer and Beñatena 1977; Roistacher 1993; Navas-Castillo and Moreno 1993a, b; Derrick and Timmer 2000; Martín et al. 2002b, 2004) and they will be largely omitted in this review, which will be focused mainly on new developments that occurred after characterization and sequencing of *Citrus psorosis virus* (CPsV) and its association with some diseases of the 'psorosis group' but not with others. It should be remarked that, although CPsV is generally assumed to be the causal agent of citrus psorosis disease, more than 80 years after the first evidence of its viral nature (Fawcett 1933, 1934) the etiology of the disease has not yet been demonstrated using Koch's postulates, mainly due to the difficulty of obtaining purified infectious virions or an infectious cDNA clone of the CPsV genome.

Historical landmarks in psorosis characterization

After psorosis was found to be graft-transmissible (Fawcett 1933, 1934), the demonstration that bark inoculum from scaled trees induced transient chlorotic flecking in young leaves of indicator plants grown in the greenhouse (Wallace 1945) was a major step forward that allowed disease diagnosis in 4 to 6 weeks instead of the 10 or more years necessary to test for bark scaling transmission (Roistacher 1991). The negative side of this advance was that in the following years several unrelated diseases were associated with psorosis based mainly on their ability to induce similar symptoms in young leaves of indicator plants.

Fawcett and Klotz (1938) proposed 2 types of psorosis, A and B, with the second inducing chlorotic ring spots in the old leaves and discoloured rings or grooves in the fruits, not observed in the A type. Later, Fawcett and Cochran (1942) showed that inoculation of healthy plants with non-lesion bark inoculum produced psorosis A symptoms (PsA), whereas inoculation with lesion bark inoculum produced rampant bark scaling and the old leaf symptoms characteristic of psorosis B (PsB). Wallace (1957) observed that plants infected with PsA were protected against challenge inoculation with PsB. This cross protection test enabled specific identification of PsA and its differentiation from other diseases of the 'psorosis group' that were unable to afford cross protection against PsB. However, based on this cross protection test, some sources of other diseases, contaminated with PsA but without bark scaling symptoms, were erroneously related with psorosis, thus re-inforcing the idea of those diseases belonging to the 'psorosis group' (Wallace 1968, 1978). Wallace (1957) proposed that PsA and PsB were 2 strains or components that would be present in all psorosis isolates. In trees propagated from psorosis-infected buds, the PsA component would initially protect against the PsB component, probably due to its more rapid increase and concentration, but later, the PsB component would become predominant in the older bark, overcoming the protective effect of PsA and causing development of the characteristic bark lesions. This process of overcoming the internal cross protection would take 10 to 15 years or longer, the time period usually necessary for bark scaling to appear in field trees, but if a healthy plant was inoculated with lesion bark pieces, the protective PsA component was not present and bark scaling started in 5 months and became rampant. However, in the absence of a known virus associated with psorosis, these hypotheses could not be tested and elucidation of the actual nature of diseases of the 'psorosis group' had to wait for decades.

Transmission of several citrus psorosis and ringspot isolates from different countries to herbaceous hosts, either by dodder (Price 1965; Desjardins et al. 1969) or mechanically (Timmer et al. 1978; Garnsey and Timmer 1980; Roistacher et al. 1980; Sarachu et al. 1988; Navas-Castillo et al. 1991), was an important step toward the purification of the hypothetical virus causing these diseases. Moreover, Garnsey and Timmer (1988) biologically cloned the agent associated with a ringspot isolate by single-lesion transfer in *Chenopodium quinoa* and then they mechanically transmitted it to *Gomphrena globosa* and to citron. When citron inoculum was graft-inoculated to sweet orange (*Citrus sinensis* (L.) Osb.) it caused typical psorosis bark scaling, indicating that i) at least some ringspot isolates contained a virus associated with psorosis bark scaling, and ii) this virus was transmissible to, and could be purified from, herbaceous hosts like *C. quinoa* and *G. globosa*.

With a reliable herbaceous indicator host available to quickly check the infectivity of different fractions from a sucrose gradient, Derrick et al. (1988a, b) demonstrated that infectivity of a ringspot isolate CRSV-4 (later re-named CPV-4) was associated with 2 fractions (the top and the bottom components), none of which was infectious alone, indicating that the putative virus associated with ringspot had a multipartite genome. An antiserum obtained to a ~48 kDa protein associated with the 2 infectious fractions and later shown to be the coat protein (CP) of CPsV (Barthe et al. 1998; Sánchez de la Torre et al. 1998) enabled detection of virus-like particles of 2 different sizes by immuno-electron microscopy, and of the ~48 kDa protein by Western blot analysis in extracts from infected plants. These findings, later confirmed with psorosis and ringspot isolates from different countries (da Graça et al. 1991; García et al. 1991a, c, 1994; Navas-Castillo et al. 1993; Navas-Castillo

and Moreno 1995), showed that psorosis and most ringspot isolates contain CPsV, whereas concave gum-blind pocket, impietratura, or cristacortis have no relationship with CPsV (da Graça et al. 1991), a conclusion also supported by biological comparison in different indicator plants (Navas-Castillo and Moreno 1993a). However, some ringspot isolates in Spain were also different from psorosis as deduced from symptom expression in citrus and *C. quinoa*, the absence of a ~48 kDa protein and the lack of cross protection against challenge with PsB (Navas-Castillo and Moreno 1993a, b). Moreover, sweet orange seedlings successively inoculated with this ringspot type and PsA or PsB displayed symptoms characteristic of both ringspot and psorosis (Moreno, unpublished). These and other ringspot isolates observed in several countries are characterized by yellow patterns in old leaves and fruits, clearly different from those of psorosis B (Fig. 3), and usually not associated with bark scaling (Broadbent 1972; Dehyar and Habashi 1974; Vogel and Bové 1981), and were later named citrus yellow ringspot (Moreno 2000a, b).

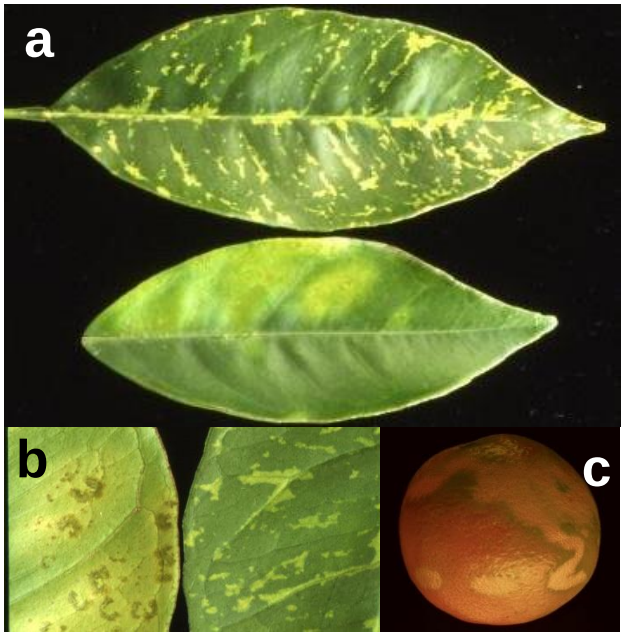

Fig. 3. Symptoms in old leaves and fruits of a navel orange tree affected by citrus yellow ringspot (CYRS), in comparison with psorosis B symptoms: a) Leaf upperside showing CYRS (upper leaf) or PsB (lower leaf) symptoms; b) Leaf underside with CYRS (right side) or PsB (left side) symptoms; c) Fruit showing color patterns characteristic of CYRS (compare with PsB-affected fruits in Fig. 1e).

The virus-like particles detected by Derrick et al. (1988a) were sinuous filaments of about 10 nm in diameter and 2 modal lengths (300 to 500 nm and 1500 to 2500 nm, in the top and bottom component, respectively), resembling spiroplasmas observed under the light microscope. Further characterization of CPsV by electron microscopy (EM), using crude extracts or purified virus preparations negatively stained with uranyl acetate (García et al. 1994) revealed highly kinked filaments of about 3 nm in diameter and a contour length approximately double than the size previously reported by Derrick et al. (1988a) using positive staining (Fig. 4). Although this unwound morphology resembles that of the bunyavirus ribonucleocapsids or the tenuivirus particles no serological relationship was found between the latter and CPsV (García et al. 1994).

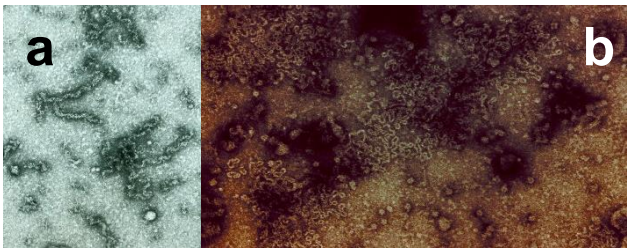

Fig. 4. Electron micrographs of the collapsed (a) and the open (b) forms of *Citrus psorosis virus* (CPsV) (Courtesy of Robert G Milne).

Barthe et al. (1998) and Sánchez de la Torre et al. (1998) obtained the first sequences of the CP gene using the CPsV isolate CPV-4 (formerly called CRSV-4) from Florida. Moreover, Sánchez de la Torre et al. (1998) found that the top component of this isolate actually had 2 ssRNAs, the CP gene being encoded by the smallest RNA, named RNA3. These advances were soon followed by sequencing the other 2 RNAs (RNA1 and RNA2) of this isolate (Sánchez de la Torre et al. 2002; Naum-Onganía et al. 2003) and later by the complete sequencing of the isolate P-121 from Spain (Martín et al. 2005). The genome organization of both isolates is shown in Fig. 5. Availability of these nucleotide sequences and of new improved polyclonal and monoclonal antibodies to the CPsV CP allowed developing new immuno-enzymatic (García et al. 1997; Barthe et al. 1998; Alioto et al. 1999; D'Onghia et al. 1998, 2000, 2001; Djelouah et al. 2000; Martín et al. 2002a; Loconsole et al. 2006; Zanek et al. 2006) and RNA-based detection procedures for CPsV (García et al. 1996, 1997; Legarreta et al. 2000; Sambade et al. 2000; Martín et al. 2004; Rosa et al. 2007; Barragan-Valencia et al. 2008; Loconsole et al. 2009, 2010; de Francesco et al. 2015; Osman et al. 2015), phylogenetic analyses among CPsV isolates and with other members of the genus *Ophiovirus* (Alioto et al. 2003; Martín et al. 2005, 2006; Achachi et al. 2015), and obtention of transgenic plants expressing CPsV genes to search for psorosis resistance (Zanek et al. 2008; Reyes et al. 2009, 2011a, b) (see below).

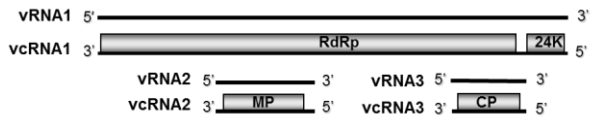

Fig. 5. Outline of the *Citrus psorosis virus* genome. Solid lines indicate the viral RNAs (vRNA) 1, 2, and 3 and the lines with blocks the complementary strands (vcRNA); the blocks indicate open reading frames (ORFs) with indication of the proteins encoded: the 24 kDa protein (24k) and the RNA-dependent RNA polymerase (RdRp) in the vcRNA 1, the movement protein in the vcRNA 2, and the coat protein (CP) in the vcRNA 3.

Molecular characterization of *Citrus psorosis virus*

Although infection of citrus plants with purified preparations of CPsV has not yet been accomplished due to the labile nature of its virions, psorosis disease and most citrus ringspot isolates described (Wallace and Drake 1968; Timmer 1974; Timmer and Beñatena 1977; Timmer et al. 1978; Wallace 1978; Timmer and Garnsey 1980; Garnsey and Timmer 1980, 1988; Sarachu et al. 1988; da Graça et al. 1991; Navas-Castillo et al. 1991, 1993; Navas-Castillo and Moreno 1993a, 1995) appear tightly associated to CPsV infection (Martín et al. 2002a, 2004), and it is generally believed that CPsV is the causal agent of those diseases. Contrarily, citrus yellow ringspot (Moreno 2000a, b), Indian citrus ringspot (Byadgi et al. 1993; Rustici et al. 2000, 2002), Bahia bark scaling disease (Passos 1965; Laranjeira et al. 2006; Nickel et al. 2007) and some atypical bark scaling disorders (Martín et al. 2002b, 2004) (Fig. 6) are not associated with CPsV infection and have a different etiology.

After sucrose gradient centrifugation and negative staining, the CPsV virions observed by EM appear as kinked filaments of approximately 3 nm in diameter with at least 2 different sizes (Milne et al. 1996). They may appear as open circular forms (O), as linear forms (L) or with an intermediate morphology (Fig. 4), with the predominant form depending on the grid preparation and staining conditions. It has been suggested that the L forms initially observed by several authors (Derrick et al. 1988a; Navas-Castillo et al. 1993) are collapsed double-stranded filaments resulting from the basic O forms after self-winding the 3 nm filaments (García et al. 1994; Milne et al. 1996). This complex morphology, resembling the ribonucleocapsid of members of the family *Bunyaviridae*, which are enveloped virions, is also observed in tenuiviruses (Francki et al. 1985), whose virions closely resemble those of CPsV, albeit tenuiviruses have a smaller CP (~33 kDa compared to 48 to 50 kDa for CPsV), are serologically unrelated with the CP of CPsV, and tenuiviruses only infect plants in the Gramineae family (García et al. 1994). This unique morphology of the virions and clear differences shown with tenuiviruses led to the classification of CPsV as the type member of the new genus *Ophiovirus*. Although the first name suggested for this new genus was Spirovirus based on the L form virions initially observed (Derrick et al. 1993), the name *Ophiovirus* was put forward and finally accepted by the International Committee on Virus Taxonomy (Milne et al. 2000) to avoid association with *Spiroplasma citri*, causing stubborn disease on citrus, and with the genus *Spiromicrovirus* used for bacteriophages affecting spiroplasmas (Milne et al. 1996). Additional ophiovirus species have been identified, most of them by their particular morphology when observed by EM. *Mirafiori lettuce big vein virus* (MiLBVV) is the causal agent of lettuce big-vein disease (Roggero et al. 2000; Lot et al. 2002) and it is transmitted by *Olpidium virulentus* (Lot et al. 2002; Sasaya and Koganezawa, 2006). *Freesia sneak virus* (FeSV) and *Lettuce ring necrosis virus* (LRNV) are transmitted by *O. brassicae* (Torok and Vetten 2002; Vaira et al. 2006) as also suggested for *Tulip mild mottle mosaic virus* (TMMMV) (Morikawa et al. 1995, 1997), whereas no report is available concerning transmission of *Ranunculus white mottle virus* (RWMV). A new proposed member of this genus has been found associated to blueberry mosaic disease (Thekke-Veetila et al. 2014).

Fig. 6. Atypical bark scaling not associated with *Citrus psorosis virus* (CPsV). a-b) Bahia bark scaling (coutesy of Cristiane J Barbosa); c) Atypical bark scaling in a CPsV-free sweet orange in French Polynesia (Martín et al. 2004; courtesy of Michel Grisoni); d) Eruptive bark lesions in a sweet orange free of CPsV in Spain (Martín et al. 2002, 2004).

Electrophoretic analysis of total RNA extracts showed the presence of ss- and dsRNA molecules in tissues infected with the psorosis isolate CPV-4 but not in healthy tissue (Derrick et al. 1991). After sequencing the genome, Northern blot analyses with (+) or (-) strand-specific probes showed that the (-) strand of the 3 viral RNAs is preferentially encapsidated (Sánchez de la Torre et al. 1998, 2002; Naum-Onganía et al. 2003). The viral RNAs (vRNAs) of the CPsV isolates CPV-4 from Florida and P-121 from Spain have been completely sequenced and both comprise 3 negative-stranded RNA segments of 8184 and 8186 nt (RNA 1), 1644 and 1645 nt (RNA 2), and 1454 and 1447 nt (RNA 3), respectively, with the same genome organization (Fig. 5) and overall nucleotide identities of 81% (RNA 1), 83.3% (RNA 2), and 85.5% (RNA 3) (Sánchez de la Torre et al. 1998, 2002; Naum-Onganía et al. 2003; Martín et al. 2005).

The complementary strand (vc) of the RNA 1 has 2 open reading frames (ORF) encoding a 24 kDa (24K) protein and a 280 kDa (280K) protein containing the motifs characteristic of viral RNA-dependent RNA polymerases (RdRp). Sequence comparisons in a conserved region in the RdRp led to the inclusion of the genus *Ophiovirus* within a distinct new family, *Ophioviridae*, among negative-stranded RNA viruses (Naum-Ongania et al. 2003; Vaira et al. 2011). Recently, the 24K protein has been localized in the nucleus and the cytoplasm and shown to interact with several citrus microRNA (miRNA) precursors (Reyes et al. 2015).

The vcRNA 2 has a unique ORF encoding a 54 kDa protein (54K). This protein localizes to plasmodesmata (PD), exhibits intercellular spread, and also facilitates the intercellular spread of GFP in trans, indicating that it has the capacity to alter the size exclusion limit of PD, a hallmark feature of viral movement proteins (MPs). The MP of MiLBVV shows similar properties (Robles Luna et al. 2013; Hiraguri et al. 2013). Preliminary data suggest that the 54K protein may also have systemic suppressor activity (Peña et al. 2010).

The vcRNA 3 encodes the CP (Sánchez de la Torre et al. 1998; Martín et al. 2005). The CP of CPsV is localized in the cytoplasm of infected *Citrus sinensis* leaf cells and it can undergo homologous interactions as revealed by fluorescent lifetime imaging microscopy and co-immunoprecipitation analysis (Peña et al. 2012). This interaction involves soluble protein in the cytoplasm, without prior formation of coat protein aggregates. Homologous interaction is expected by the structural function of the CP and it is supported by EM images of ophiovirus particles showing CP subunits embracing the RNA like a ring (Robert G Milne, personal communication). The CP of MiLBVV also localizes in the cytoplasm of infected cells and undergoes homologous and heterologous interactions (Peña et al. 2012). Indeed the CPs of CPsV and MiLBVV can interact in vivo upon co-expression. This interaction does not seem to play a role in nature since the 2 viruses do not occur in the same host; however, it could be important for other ophioviruses sharing the same host. In the presence of the 54K protein, no re-localization of CP to PD, nucleus, or microtubules (MT) was observed. However, it was found that the CP interacts with the MP of CPsV in the cytoplasm, suggesting a potential role of CP in ophiovirus movement (Robles Luna et al. 2013).

Detection, characterization, and genetic variation of *Citrus psorosis virus* isolates

For many years psorosis infection was diagnosed by biological indexing on young sweet orange seedlings: firstly, based on transient chlorotic flecking and spotting development in young leaves, sometimes preceeded by a shock reaction with leaf shedding and necrosis of the first flush (Fig. 2), and later, using cross protection against challenge inoculation with a PsB isolate to avoid confusion with similar symptoms caused by other agents in young citrus leaves (Wallace 1945, 1957, 1978; Roistacher 1980, 1991, 1993).

After psorosis was associated with the presence of 2 components with a ~48 kDa protein, later shown to be the CP of CPsV (Barthe et al. 1998; Sánchez de la Torre et al. 1998), a first antiserum obtained by Derrick et al. (1988) enabled detection of the putative virus associated with psorosis by Western blot analysis and by immuno-electron microscopy (da Graça et al. 1991; Navas-Castillo et al. 1993; Navas-Castillo and Moreno 1995; García et al. 1991a, c, 1994). This was followed by obtention of a more specific antiserum allowing immuno-enzymatic detection of the virus (García et al. 1997; D'Onghia et al. 1998, 2000, 2001; Loconsole et al. 2006), as well as monoclonal antibodies that improved sensitivity and allowed differentiation of CPsV isolates (Alioto et al. 1999, 2000, 2003, 2008; Djelouah et al. 2000; Martín et al. 2002a, 2004; Zanek et al. 2006). Serological analysis of 53 psorosis field sources from Campania (Italy) allowed detection of 9 different serogroups with at least 10 different epitopes (Alioto et al. 2003). An important conclusion emerging from these data is that ELISA diagnostics based on the use of a single monoclonal antibody may be unreliable, therefore, polyclonal antibodies or a proper mixture of monoclonal antibodies should be used to avoid false negatives.

Similarly, availability of partial nucleotide sequences of the CPV-4 isolate enabled sensitive detection of CPsV by different hybridization and RT-PCR protocols (García et al. 1996, 1997; Barthe et al. 1998; Legarreta et al. 2000; Martín et al. 2004; Rosa et al. 2007; Barragan-Valencia et al. 2008; Loconsole et al. 2009, 2010; de Francesco et al. 2015; Osman et al. 2015). These new detection methods allowed the association of psorosis disease, as diagnosed by symptom expression in the field and on indicator plants including cross protection against PsB, with the presence of CPsV as detected by different CP- or RNA-based procedures (Martín et al. 2004). They also opened the way to sequence comparisons to analyze genetic variation within and among CPsV isolates.

Single-strand conformation polymorphism (SSCP) analysis (Rubio et al. 1996) of clones from the same genomic segment RT-PCR-amplified from different CPsV isolates showed that in most of them the viral population consisted of a predominant sequence with a few minor variants genetically close to the main sequence (Velázquez et al. 2012). This procedure also allowed rapid differentiation of some CPsV isolates, as well as study of the interaction between isolates by analysing the changes in the population structure in plants doubly inoculated. Using this approach, it was observed that in plants inoculated first with the PsA isolates P-121 or P-126, or with the PsB isolate PB-143, and 3 months later challenge-inoculated with another isolate in all possible combinations, the SSCP profile of the CP gene in doubly inoculated plants 2 years later showed only the first isolate inoculated. That is, the first isolate apparently excluded accumulation of the second isolate, indicating that not only does PsA cross protect against challenge

inoculation with PsB as previously shown by Wallace (1957), but PsB also protects against PsA. This latter protection cannot be monitored by symptom observation because PsA does not induce symptoms in twigs and old leaves, and symptoms on young leaves are similar for both psorosis types (Guerri, unpublished). Simultaneous co-inoculation of healthy plants with different pairs of the above 3 isolates allowed accumulation of the 2 isolates inoculated, as detected by a composite SSCP profile in the inoculated plants; however, the accumulation of each isolate was variable depending on the combination, with P-121 accumulating more and P-126 less than PB-143.

SSCP analysis also allowed differentiation of the PsA and PsB components separated from the same psorosis affected tree by inoculating healthy sweet orange seedlings with non-lesion or with lesion bark inoculum, respectively (Fawcett and Cochran 1942). While comparisons of different homologous regions of the RNAs 1 and 3 from the PsA and PsB components showed identical SSCP profiles, segments of the RNA 2 showed distinct SSCP profiles allowing identification of either component (Velázquez et al. 2012). Alignment of the RNA 2 sequences from different PsB sources did not allow identification of any sequence specifically associated with isolates of the PsB type.

A first sequence comparison among 19 Italian isolates showed limited variation in the CP gene, with the 3' proximal region being more variable than the 5' proximal region. However, these isolates widely differed from the CPV-4 isolate from Florida (Alioto et al. 2003). Further comparison of CPsV isolates from Spain, Italy, California, Florida, and Argentina in 3 coding regions located in the RNAs 1, 2, and 3, disclosed 2 populations: one including isolates from Spain, Italy, California and Florida, and the other comprising the Argentinean isolates (Martín et al. 2006). Again, the isolate CPV-4 included for comparison clustered separately from these 2 populations, suggesting its belonging to a third population. The low ratio between non-synonymous and synonymous nucleotide substitutions indicated negative selection for amino acid changes, particularly in the CP gene. Exchange of genomic segments, as indicated by incongruent phylogenetic relationships in different genomic regions, may have also contributed to CPsV evolution. In summary, CPsV has evolved at least 3 genetically differentiated populations that likely were shaped by the combined effects of selection for amino acid conservation, genetic exchange between sequence variants, and gene flow between countries (Martín et al. 2006). Similar conclusions have been reported based on analysis of a Moroccan population of CPsV (Achachi et al. 2015).

Virus-host interactions

Our understanding of CPsV-citrus interactions is still limited. Several lines of evidence indicate that, contrasting with other citrus viruses like *Citrus tristeza virus* (CTV) (genus *Closterovirus*) or Citrus vein enation virus (CVEV) (a likely member of the genus *Enamovirus*), CPsV is not restricted to phloem-associated cells, but invades other parenchymatous cells: i) chlorotic flecks and spots in young leaves are not associated with veins or veinlets, but most often appear in interveinal regions, ii) the yield of infectious virions is highest when they are purified from symptomatic leaf regions, iii) bark scaling, the most characteristic symptom of psorosis, is caused by suberization of parechyma layers in the bark, without affecting phloem tissues, indicating that the virus invades the cortical parenchyma (Schneider 1969), iv) CPsV remained in callus cultures obtained from plants infected with PsA, PsB, or ringspot isolates and could be transmitted to healthy plants upon graft inoculation with infected callus pieces, whereas calli obtained from shoot internodes infected with phloem-restricted viruses like CTV or CVEV were not infectious (Duran-Vila et al. 1991; Navas-Castillo et al. 1995), v) efficient elimination of psorosis by shoot-tip grafting in vitro required using very small shoot tips from heat treated plants (Navarro et al. 1980), suggesting that the virus is present very close to the meristem, in a region where phloem tissues are not yet differentiated, and vi) graft-inoculation of sweet orange seedlings with psorosis-infected bark patches followed by inoculum removal at different times (1 through 14 days after grafting), resulted in plant infection after 5 to 7 days (Reyes et al. 2009), a period in which vascular connections between the inoculum and the receptor plant have not yet developed. A similar experiment with CTV resulted in receptor plants infected only when inoculum was removed after 14 or more days (Moreno, unpublished).

Tropism of psorosis A and B variants

After the PsA and PsB components of psorosis isolates could be identified by distinct SSCP profiles of their RNA 2, different types of tissues were analyzed to examine the distribution of these 2 sequence variants (Velázquez et al. 2012). It was observed that i) PsA isolates contain both PsA- and PsB-associated sequences, albeit the second type is usually at low frequency, and viceversa, the PsB isolates also contain small amounts of PsA variants, ii) in old leaves with yellow blotches and gummy pustules and in blistered shoots, characteristic of psorosis B, the predominant sequence variant is that associated with PsB, and iii) the PsB variant also predominates in bark lesions of the trunk. Wallace (1957) suggested that in trees with PsA symptoms, delay in bark scaling appearance would be due to the faster accumulation or movement of the PsA component that would temporarily prevent accumulation of the PsB component by internal cross protection.

Since differences between both psorosis types are associated with changes in the RNA2, the tropism observed suggests potential association of the 54K protein, the MP, with bark scaling and PsB symptoms, although other viral proteins might be also involved in pathogenicity (Velázquez et al. 2012).

Interactions between PsA and PsB components in co-inoculated plants

The distinct SSCP profiles of the PsA and PsB variants of CPsV also enabled the dissection of the interactions of these 2 components in doubly inoculated plants. Plants inoculated with the PsA or the PsB sub-isolate separated from a psorosis-infected tree and then challenge-inoculated with the PsB or the PsA component, respectively, showed the symptoms and SSCP profile characteristic of the first component inoculated, even 4 years after inoculation, thus supporting the notion that CPsV isolates are able to cross protect at least against challenge with other isolates of the same group (Martín et al. 2006) and that interference between PsA and PsB components operates in both ways (Velázquez et al. 2012). It has been observed that CPsV triggers, and is the target of, the RNA silencing mechanism of citrus plants (Velázquez et al. 2010; Reyes et al. 2011). However, whether the observed protection in pre-inoculated plants operates by small RNAs derived from the first isolate interfering with the accumulation of viral RNA of the second (Ratcliff et al. 1999), or by some protein-mediated interaction (Folimonova et al. 2010, 2014) is presently unknown.

When the PsA and PsB sub-isolates were simultaneously co-inoculated in sweet orange seedlings, the SSCP profile was a composite of the profiles characteristic of each component. The PsB-associated profile was initially more intense in the trunk than in the leaves, but 6 months after inoculation this profile became predominant in all tissues and the plants started showing psorosis B symptoms (Velázquez et al. 2012).

Overall, the results on the tropism of the PsA and PsB components and on co-inoculation experiments support Wallace's hypothesis (Wallace 1957) in that: i) psorosis isolates contain both the PsA and PsB components even when only PsA symptoms are observed, ii) the PsB-associated variant accumulates preferentially in the trunk bark, iii) the PsB-associated variant is predominant in tissues starting to show PsB symptoms in PsA-affected plants, and in all tissues of the plants showing PsB symptoms, and iv) pre-inoculation with the PsA sub-isolate and challenge-inoculation with PsB results in exclusion of the later component and complete protection against PsB symptoms. Contrarily, they disagree with Wallace's suggestion that delay of bark scaling appearance in trees bud-propagated or graft-inoculated from PsA-affected trees would be due to the higher multiplication and/or faster movement of the PsA component that would prevent accumulation of the PsB component. The new data suggest that the symptoms observed after inoculation with tissue taken from a PsA- or a PsB-affected tree likely depend on the ratio of the PsA- and PsB-associated sequence variants present in the inoculum, rather than on the fitness of either variant (Velázquez et al. 2012). Delayed appearance of bark scaling in trees bud-propagated from PsA-affected trees (10 to 15 years) could be due to the low frequency of the PsB-associated sequence variant in green symptomless tissues of the budwood source trees. With time, and due to the preferential accumulation of the PsB variants in the trunk bark, these variants would become predominant in these tissues and incite progressive bark scaling.

Symptoms and virus accumulation in infected plants

The molecular mechanisms involved in psorosis symtom expression are presently unknown. However, early experiments showed that symptoms incited in young indicator plants were milder or null in plants grown at high temperature (32 to 38 ºC maximum) in comparison with plants grown at low temperature (24 to 27 ºC maximum). For this reason, biological indexing of psorosis is currently done at 24 to 27 ºC (Roistacher 1980, 1991, 1993). These observations also led to the development of thermotherapy as a procedure to obtain psorosis-free budwood from psorosis-infected plants (Calavan et al. 1972; Roistacher and Calavan 1972, 1974). Moreover, heat sensitivity of psorosis and other diseases of the former 'psorosis group' was used to improve efficiency of shoot-tip grafting in vitro to obtain virus-free plants by defoliating bud-propagated infected plants in the greenhouse and incubating them at 32 ºC to induce new flush under warm conditions (Navarro et al. 1980).

Monitoring by ELISA and Northern blot hybridization of the virus titer of psorosis-infected plants incubated at 26/18 ºC (day/night) or at 32/26 ºC (day/night) showed that virus titer paralleled symptom intensity (Velázquez et al. 2010). The plants incubated at cooler temperature displayed a shock reaction with shoot necrosis in the first flush and moderate to intense chlorotic leaf flecking and spotting in the following flushes, whereas those incubated at warmer temperature did not show the shock reaction and young leaf symptoms were milder. The amount of CPsV-derived small RNAs (CPsV-sRNAs) was slightly higher at the warmer temperature and the ratio CPsV-sRNA/vRNA was higher at 32/26 ºC than at 26/18 ºC, suggesting that symptom intensity is associated to virus accumulation and that temperature decreases symptom intensity by enhancing the RNA silencing response of the citrus plants and thus reducing virus accumulation. This effect of temperature on the intensity of the RNA silencing response of citrus plants may be also behind the reduced symptom expression and thermotherapy elimination of other citrus viruses (Calavan et al. 1972; Roistacher and Calavan 1974).

Molecular interactions between host and virus factors

The molecular basis for the virus-host interactions described previously is largely unknown and only recently some data on the cellular localization of the viral proteins and their potential interactions with host factors have been obtained.

As indicated above, the 24K protein encoded in the vcRNA 1 is localized in the nucleus and the cytoplasm and it interacts with several citrus microRNA (miRNA) precursors (Reyes et al. 2015). The miRNA precursors pre-miR156a and pre-miR171a of citrus plants co-

immunoprecipitated with 24K, indicating their interaction, a phenomenon that was associated with high level of the pre-miRNAs, reduced levels of the cognate miRNAs and over-expression of their target mRNAs in CPsV-infected leaves. These findings suggest that the altered processing of these pre-miRNAs might be due to a direct or indirect interaction with the 24K protein, a result that is consistent with the nuclear localization of both the pre-miRNAs and the 24K protein of CPsV. Some up-regulated miRNA targets like *Squamosa promoter-binding protein-like* 9 and 13 and *Scarecrow-like* 6 (SCL6) have been associated with abiotic stress tolerance and play a regulatory role in the resistance to the bacterial pathogen *Pseudomonas syringae* in *Arabidopsis thaliana* and to *Tobacco mosaic virus* (TMV) infection in *Nicotiana benthamiana*. Up-regulation of SCL6 could also negatively regulate chlorophyll biosynthesis and induce chlorosis.

The 54K protein encoded by the vcRNA 2 was located inside the PD channel, which is consistent with a role in cell-to-cell movement (Robles Luna et al. 2013). The negative polarity of the CPsV genome has impaired investigating the function of this protein using reverse genetics, but in trans-complementation assays, 54K functionally complemented cell-to-cell movement-defective *Potato virus X* (PVX) and TMV mutants, showing that this protein is the MP of CPsV. Interestingly, the fusion protein eGFP:54K accumulates along the microtubules, indicating direct or indirect interaction with these filaments. This can be associated with the finding that viral replication complexes of TMV (trans-complemented by the 54K) are anchored to the microtubules and at early stages of infection they are targeted to PD, indicating a putative function of the 54K protein in that localization.

Finally, the CP of CPsV encoded by the vcRNA 3 is localized in the cytoplasm of infected cells and it undergoes homologous an heterologous interactions (Peña et al. 2012). In the presence of the 54K protein, no re-localization was observed of the CP to PD, nucleus or MT, the typical MP localizations. However, it was found that the CP did interact with the 54K protein in the cytoplasm, suggesting a potential role of the CP in ophiovirus movement (Robles Luna et al. 2013).

Sensitivity of different citrus genotypes to Citrus psorosis virus

The susceptibility or resistance of different citrus genotypes to CPsV has not yet been established properly. From field tree observations, it has been known for years that sweet oranges, mandarins (*C. reticulata* Blanco), and grapefruits (*C. paradisi* Macf.) are sensitive to psorosis bark scaling, while other species like sour orange (*C. aurantium* L.), sour lemons (*C. limon (L.) Burn. f.*), or rough lemon (*C. jambhiri* Lush.) do not show bark scaling but infected plants display psorosis-like young leaf symptoms (Roistacher 1980). Since other graft-transmissible diseases of citrus also cause similar young leaf symptoms, and bark scaling caused by agents other than psorosis have been observed (Martín et al. 2002b, 2004), psorosis infection of unknown sources need to be confirmed by indexing on sensitive indicator plants and a cross protection test against PsB (Roistacher 1980, 1991, 1993; García et al. 1991b), but these tests were omitted in many reports. Also, symptomless infections of some genotypes with CPsV might go unnoticed.

Graft-inoculation of 63 cultivars and hybrids of *Citrus* and related genera [*Citrus* (37), *Fortunella* (6), *Microcitrus* (5), *Atalantia* (2), *Afraegle* (1), *Clausena* (1), *Eremocitrus* (1), *Pleiospermium* (1), *Poncirus* (1), *Severinia* (1), *Swinglea* (1), and hybrids (7)] propagated on rough lemon rootstock with CPsV showed that most of them displayed symptoms and gave a positive ELISA reaction to CPsV, 2 genotypes (*Microcitrus inodora* (Bail.) Swing. and *Fortunella hindsii* Champ. ex Benth.) remained symptomless but gave high ELISA values, suggesting tolerance to CPsV, whereas 6 others were ELISA negative, suggesting at least partial resistance to the virus (Velázquez et al. 2015). Inoculation of Cleopatra mandarin (*C. reshni* Hort. ex Tan.), trifoliate orange (*Poncirus trifoliata* (L.) Raf.), and Carrizo citrange (*C. sinensis* × *P. trifoliata*) seedlings, which in the previous exploratory experiment were CPsV negative by ELISA, with 3 distinct CPsV isolates and monitoring CPsV infection by symptom expression and by reverse transcription quantitative real time PCR (RT-qPCR) of the RNA 3, revealed a lower ratio of infected plants and a delay in symptom appearance and in virus accumulation in comparison with Pineapple sweet orange, a sensitive variety used as control. This resistance was different depending on the isolate and citrus genotype. Propagation of these genotypes on a CPsV-inoculated sweet orange rootstock caused a disorder with bark necrosis at the bud union line between the scion and the rootstock. These findings suggest that the high viral load in the susceptible rootstock induces a hypersensitive-like reaction with cell death that prevents or delays virus infection.

Psorosis transmission and epidemiology

The main way of psorosis dispersal is by propagation of infected buds. The long period necessary for bark scaling to appear (at least 10 to 15 years) likely allowed, unbeknown to growers, psorosis-infected trees to be selected as budwood sources, thus contributing to the high incidence of this disease in the old citrus lines of certain areas like the Mediterranean basin.

Experimentally, psorosis and some ringspot isolates have been transmitted to other hosts by dodder (Price 1965; Desjardins et al. 1969) and by mechanical inoculation (Timmer et al. 1978; Garnsey and Timmer 1980, 1988; Roistacher et al. 1980), but these procedures are epidemiologically irrelevant.

Potential transmission of psorosis through trifoliate orange and Carrizo or Troyer citrange seeds was reported (Bridges et al. 1965; Childs and Johnson 1966; Pujol and Beñatena 1965; Pujol 1966; Campiglia et al. 1976), however, it was based on the observation of young leaf

symptoms and no bioindexing was performed to confirm that the observed symtoms were due to psorosis. Considering that bark scaling has never been observed on these genotypes and that they show partial resistance to different CPsV isolates (Velázquez et al. 2015) it is unlikely that the symptoms observed actually corresponded to psorosis. So far, virus transmission via citrange seeds has been confirmed only for *Citrus leaf blotch virus* (Guerri et al. 2004), the causal agent of Dweet mottle disease that incites transient young leaf symptoms on Dweet tangor (*C. tangerina* Hort. ex Tan. × *C. sinensis*) (Roistacher and Blue 1968; Vives et al. 2008).

Several observations suggesting natural spread of psorosis by a vector have been reported in Texas and in Argentina. In Texas, bark scaling of grapefruit seedlings or propagations from seedling plants was observed at a slow rate, but limited vector-transmission experiments using insect species visiting citrus in the area were unsuccessful (Timmer 1974; Timmer and Garnsey 1980). After most ophioviruses were shown to be transmitted by fungi within the genus *Olpidium*, Palle et al. (2005) RT-PCR-analyzed for CPsV the zoospores from an *Olpidium*-like fungus infecting the roots of healthy or psorosis-infected grapefruit trees in Texas. A 136 bp fragment with 90% identity with the CPsv RNA 1 was amplified from the zoospores obtained from the infected, but not from the healthy, roots. However, limited trials adding viruliferous zoospores to healthy seedlings failed to transmit CPsV.

In Argentina, the disease caused rampant bark lesions in sweet oranges and grapefruits and the rate of dispersal was fast (Pujol and Beñatena 1965; Timmer and Beñatena 1977). Many bark-scaled trees were seedlings or propagations from nucellar plants, presumably virus-free, and an aerial vector for the disease was suspected (Beñatena and Portillo 1984). In a field experiment, sweet orange seedlings, planted close to a Pira Lima sweet orange block heavily affected by bark scaling, started showing psorosis-like young leaf symptoms and a shock reaction in young shoots 3 years after planting, with most newly infected seedlings being close to the border shared with the Pira Lima block. Control plants kept under screen did not show symptoms. Continuous monitoring of the insects visiting the affected plants showed that the most frequent visitor was *Aphis citricola*, followed by *A. gossypii*, and *Toxoptera citricida* (Beñatena and Portillo 1984). In further transmission experiments (Portillo and Beñatena 1986) the authors claimed psorosis transmission by *T. citricida*, *T. aurantii*, *Toxoptera* spp., and *A. citricola*, however, bioindexing proofs or CPsV detection by serological or RNA-based procedures to check that the disease transmitted was psorosis have not been performed. Moreover, direct evidence that CPsV is actually acquired by aphids and transmitted to receptor plants is still lacking. In conclusion, in spite of the evidence for natural spread of a psorosis-like disease occuring in some citrus areas, presently, the only way proven for CPsV dispersal is propagation of infected buds.

Control of psorosis

Since psorosis dispersal in most citrus areas occurs only by the use of infected budwood, the simplest way to control this disease is using psorosis-free buds for new plantings or for topworking old plantings, by launching a plant and budwood certification program (Navarro et al. 2002). Budwood source plants free of psorosis have been obtained by heat therapy (Calavan et al. 1972; Roistacher and Calavan 1972, 1974) or by shoot-tip grafting in vitro (Navarro et al. 1975, 1980). A complementary measure to avoid potential introduction of naturally-spreading psorosis variants is establishing a quarantine system for introduction of new varieties (Navarro et al. 1984; Navarro 1993), particularly when budwood is to be imported from areas where natural disease dispersal occurs. In these areas, the use of psorosis-free certified buds in new plantings would need supplementary measures like vector control once the vector species will be unequivocally identified, or in the long term, the use of resistant varieties.

A major constraint for the use of natural resistance is that most if not all commercial varieties of sweet orange, mandarin, and grapefruit are sensitive to CPsV. So far partial resistance, isolate-dependent, has been detected only in trifoliate orange and citrange and in Cleopatra mandarin (Velázquez et al. 2015), which makes it difficult to incorporate this resistance into commercial varieties by conventional breeding. An alternative approach to obtain CPsV-resistant varieties would be the production of transgenic plants expressing CPsV genes able to induce silencing of viral RNA upon CPsV infection. Reyes et al. (2009) obtained transgenic *N. benthamiana* plants carrying intron-hairpin constructs of the *cp* (ihpCP) or the *54k* (ihp54) genes. Analysis of CPsV-derived sRNAs and of vRNA indicated a higher degree of silencing with the ihpCP construct, not only against the homologous CPsV isolate (90-1-1 from Argentina) but also against the distant isolate CPV-4. Similarly, different lines of transgenic sweet orange plants expressing ihp24, ihp54, or ihpCP from the 90-1-1 CPsV isolate were obtained and tested for protection against infection with the homologous isolate (Reyes et al. 2011b). While lines expressing ihp24 or ihp54 were susceptible to CPsV infection with little or no protection being afforded, several lines derived from the CP displayed partial or full resistance in 2 different experiments. In contrast, transgenic sweet orange plants expressing the *24k*, *54k*, or *cp* genes from the CPV-4 isolate were susceptible to infection either with the homologous isolate or with a PsB isolate from Argentina (Reyes et al. 2011b). These results suggest that transgenic resistance to at least some CPsV isolates is possible in at least some sweet orange cultivars.

Contrasting with vector-transmitted viruses like CTV, cross protection is not an adequate measure to control psorosis disease, in spite of a recent suggestion in favor of its use with CPsV (Achachi et al. 2014). Firstly, in most areas the virus is dispersed only through infected budwood, and therefore, using certified CPsV-free plants

or buds is enough to guarantee disease absence in the new plantings; and secondly, in areas where natural disease spread occurs, it would be necessary to use asymptomatic protective CPsV isolates, but no isolate of these characteristics has yet been reported. It is more likely that new bitechnological approaches will help circumventing the specific problem of these areas.

Concluding remarks

Our knowledge of psorosis and CPsV has clearly increased in the last 3 decades, but 120 years after the first report on psorosis there are more questions than answers on this disease and its presumed causal agent. A first question is whether psorosis is actually caused only by CPsV. Although single-lesion cloning of a psorosis (ringspot type) isolate in *C. quinoa* and then mechanical transmission to *G. globosa*, Etrog citron and sweet orange induced in this later host the symptoms characteristic of this isolate and no other viral particles have ever been observed by EM, the possibility of a second non detected agent co-transmissible with CPsV cannot be completely ruled out. The labile nature of the CPsV virions and its tripartite genome further complicates obtaining concentrated, highly purified infectious virion preparations and successful inoculation to citrus. With viruses having monopartite positive-stranded ssRNA genomes, development of an infectious cDNA clone of the full genome and agro-infiltration in an adequate host may circumvent purification problems to prove disease etiology (Vives et al. 2008), a procedure that is far more complicated with negative-stranded viruses, particularly those with a segmented genome (Neumann et al. 2002).

After unequivocally proving that CPsV is the only causal agent of psorosis, the role of the different viral proteins on pathogenicity should be investigated. The 24K protein interferes with the processing of several important pre-miRNA molecules thus causing up-regulation of several host factors involved in abiotic stress tolerance, and in regulation of the resistance to pathogens and of chlorophyll bisynthesis (Reyes et al. 2015). Is this viral protein responsible for chlorotic leaf patterns in affected citrus trees? Is the transient nature of psorosis young leaf symptoms due to the progressive reduction in the CPsV load observed in leaves as they mature? Polymorphisms identified in the *54k* gene suggests that the 54K protein might be related with expression of the PsB symptoms, but is it responsible for all or just for some of the symptoms observed? Why does the shock reaction usually occur only in the first flush after inoculation? Is this related with a very high viral load in the first flush inciting a hypersensitive reaction, whereas virus accumulation in the following flushes is kept to a lower level after RNA silencing being set up? Are either the 24K or the 54K proteins responsible for this symptom? How is the PsB sequence variant of the 54K protein associated with permanent chlorotic blotching in old leaves, twig blisters, or trunk bark scaling? Expression of the 54K from PsA and PsB isolates in sweet orange seedlings using a viral vector (Agüero et al. 2012) might help in elucidating part of these questions. An early function of the CP in virus infection can be suspected since silencing this gene in transgenic plants expressing ihpCP inhibits CPsV accumulation, whereas the ihp54K and ihp24K constructs do not impair virus infection. Knowledge on the function of the different viral-encoded proteins may help designing adequate transgenes to obtain better resistance to CPsV in different citrus varieties.

The question of natural dispersal of psorosis by a vector is basically unsolved 50 years after the first observations indicating field spread. Availability of ELISA, hybridization, RT-PCR, or RT-qPCR for CPsV may enable detection of potential vectors by testing different organisms for CPsV acquisition. Then, transmission tests could be performed with those accumulating a higher viral load. A first step in this direction was given to detect CPsV in an *Olpidium*-like fungus found in citrus roots (Palle et al. 2005). CPsV detection procedures should also be used to search for potential non-citrus hosts that might be efficient reservoirs for CPsV dispersal. Understanding virus epidemiology in areas where natural disease spread occurs is critical to set up control protocols.

In conclusion, after so many years of darkness in our knowledge of psorosis, discovery and partial characterization of CPsV has just started to throw a pale light at the end of the tunnel.

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