Current status of the *Citrus leprosis virus* (CiLV-C) and its vector *Brevipalpus phoenicis* (Geijskes)

Situación actual del virus de la leprosis de los cítricos (CiLV-C) y su vector *Brevipalpus phoenicis* (Geijskes)

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ABSTRACT

The *Citrus leprosis virus* CiLV-C is a quarantine disease of economic importance. Over the past 15 years, this disease has spread to several countries of Central and South America. Colombia has about 45,000 hectares of citrus planted with an annual production of 750,000 tonnes. The CiLV-C has only been detected in the departments of Meta, Casanare and recently Tolima. Meta has 4,300 hectares representing 10% of the national cultivated area, and Casanare, where CiLV-C appeared in 2004, has no more than 500 ha planted with citrus. The presence of the citrus leprosis virus in Colombia could affect the international market for citrus, other crops and ornamental plants with the United States and other countries without the disease. The false spider mite *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae) is the main vector of the CiLV-C. Disease management is based on control programs of the vector and diminishing host plants. Chemical mite control is expensive, wasteful and generates resistance to different acaricides. This paper provides basic information on CiLV-C and its vector, advances in diagnosis and methods to control the disease and prevention of its spread.

Source photographs: Figs. 1-4: León M., G.

Key words: diagnosis, leprosis, vector mite, control.

RESUMEN

La leprosis de los cítricos CiLV-C es una enfermedad de importancia económica y cuarentenaria. Durante los últimos 15 años se ha comprobado su dispersión por varios países del Centro y Sur América. Colombia posee aproximadamente 45.000 hectáreas cultivadas en cítricos, con una producción anual de 750.000 toneladas. La CiLV-C únicamente ha sido detectada en los departamentos del Meta, Casanare y recientemente en Tolima. El Meta, con 4.300 hectáreas representa el 10% del área nacional cultivada y Casanare, en donde se confirmó la presencia del CiLV-C en el año 2004, no posee más de 500 ha sembradas con cítricos. La presencia de la leprosis de los cítricos en Colombia, puede afectar el mercado internacional, así como el de otros productos agrícolas y plantas ornamentales con Estados Unidos y otros países que no poseen la enfermedad. El ácaro rojo plano *Brevipalpus phoenicis* (Geijskes) (Acari: Tenuipalpidae) es el principal transmisor del CiLV-C. El manejo de la enfermedad, se basa en programas de control del vector y la disminución de plantas hospederas. El control químico del ácaro, es costoso, dispendioso y genera resistencia. El presente trabajo provee información básica sobre CiLV-C y su vector, avances en diagnóstico, métodos para control y prevención de diseminación de la enfermedad.

Palabras clave: diagnóstico, leprosis, ácaro vector, control.

Introduction

The *Citrus leprosis virus* (CiLV-C) is a quarantine and economically important disease, which represents millions of dollars in damage to citrus crops in countries where it has been established, affecting mainly the orange and mandarin and is reported only in South America and Central America. During the past 15 years, it has caused economic losses in Argentina, Brazil, Paraguay, Uruguay, Venezuela, Costa Rica, Panama and Honduras. The disease was recently reported in Guatemala, Peru, Bolivia and Colombia (Locali *et al*., 2003; Saavedra *et al*., 2001; Mejía *et al*., 2005; Gómez *et al*., 2005; León *et al*., 2006).

According to Rodrigues *et al*. (2001) and Freitas *et al*. (2004), CiLV-C is considered the most important viral disease in the Brazilian citrus industry because the costs of controlling the mite vector reach about $100 million dollars per year. Besides the importance of the disease to the Brazilian citrus industry, its global importance has grown in recent years due to the spread of the virus to other countries in Central and South America (Kitajima *et al*., 2004; Freitas *et al*., 2005).

The importance of CiLV-C is that the disease severely affects production and is considered a quarantine disease, therefore there are restrictions for international marketing of some agricultural products, especially in countries of North America, Europe and Asia which do not have the disease.

Colombia has about 45,000 ha planted with citrus with an annual production of 750,000 t. The citrus leprosis virus

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was detected in 2003 in Yopal, Casanare; by 2005 the disease spread to all of Meta and Casanare (Leon *et al*., 2006; Becerra *et al*., 2007). The CiLVC has only been reported in Meta and Casanare. The citrus area planted in Meta is approximately 4,300 ha, which represents 10% of the national cultivated area. Casanare, where CiLV-C appeared in 2004, has no more than 500 ha planted with citrus. The estimated production for Meta is $180.000 million pesos per year, providing reason to develop control programs (MADR, 2002).

Knowing the importance of the disease to Colombia, the objective of this study is to publish research advances concerning the virus and its vector, as a basis for future research and control methods striving for economic impact prevention as well as reduction in the spread of the disease in Colombia.

History and distribution of the disease

Childers *et al*. (2003) and the USDA, 2004 say that the disease was first reported in Florida, United States in 1901 and was called “scale bark”. The disease almost destroyed the citrus industry in Florida before 1925, but has not reappeared since 1968, possibly due to the use of sulfur to control mites, frequent freezes and hurricanes that devastated large areas planted in orchards. In South America, it was first identified in 1920 in Paraguay (Spegazzini, 1920), where it was called “lepra explosiva”. In a short period, the disease was observed in Argentina; and in Sao Paulo, Brazil, it was reported in 1931 on leaves of the ‘Bahiana’ variety orange and then in several sweet oranges (Bastianel *et al*., 2006).

During the 1930s and 1940s, it was suggested that leprosis could be caused by a virus. However, the etiology of the disease was confirmed by electron microscopy just after 1972 with the observation of virions in the symptomatic tissue (Kitajima *et al*., 1972), and transmissions to alternate hosts through mechanical inoculations (Colariccio *et al*., 1995). Kitajima (1972), described the leprosis virions in citrus leaves and determined that this virus may be cytoplasmic (CiLV-C) or nuclear (CiLV-N). The cytoplasmic virus is widely distributed in affected countries, unlike the nuclear virus which is uncommon in citrus groves (Kitajima *et al*., 2004; Kitajima, 2005).

The citrus leprosis is restricted to countries in Central and South America (Rodrigues *et al*., 2001), in Mexico, the presence of the disease was confirmed in Chiapas in July 2005 (NAPPO, 2005). Citrus leprosis was reported in Costa Rica by Araya (2000), in Panama by Saavedra *et al*. (2001), in Guatemala by Mejía *et al*. (2005) and in Bolivia by Gómez *et al*. (2005).

In Colombia, the citrus leprosis disease was observed in 2003 in Yopal, Casanare, and identification was officially confirmed in 2004 (León *et al*., 2006). During 2004 and 2005, leprosis was observed in several citrus orchards in Meta and Casanare. ICA has found the citrus leprosis disease in Ibague, Tolima, but this record has not been published (Bastianel *et al*., 2010). Although the red flat mite *B. phoenicis* has been registered in several areas of the country for more than three decades, the virus had not been recorded previously in Colombia (León *et al*., 2005).

Characteristics and disease diagnosis

Kitajima *et al*. (1972) reported the occurrence of viral particles in the tissues of leprosis symptomatic leaves but particles in the surrounding asymptomatic areas were not found. This indicates that the virus does not spread systemically in the host, which was confirmed by RT-PCR tests (Locali *et al*., 2004). The dispersion of the disease in the plant occurs through movement of viruliferous mites within the plantation and is a consequence of their feeding habits (Rodrigues *et al*., 2001).

The citrus leprosis virus is able to cause symptomatic injury in leaves, branches and fruits of citrus. The sweet orange trees are the most susceptible; tangerines and grapefruits have a medium susceptibility to the disease. Acid oranges, acid limes, lemons and tangelos show signs of lower susceptibility to the citrus leprosis virus (Rodrigues *et al*., 2001; Locali *et al*., 2004).

Symptoms on leaves

Leaf lesions are superficial and visible on both sides, but extremely variable, rounded or oval, often circular with a 5 to 12 mm diameter. Initially, lesions are present as light green spots surrounded by a yellow ring (Fig. 1). Typical lesions are chlorotic or necrotic; vary in color from light yellow to dark brown when they are fresh or when the injury is old respectively.

In older lesions, it is possible to observe a central dark spot with concentric halos which over time become dry. Circular spots may occur with the dry centers or stains of different sizes and shapes that extend, invading most of the leaf. Affected leaves eventually fall off.

Symptoms in branches

Injuries are located preferentially in young stems of fruiting branches. The first symptoms are yellowing circular spots, pale green or brown, with sizes varying from 0.5 to 1.0 cm. that look chlorotic, small lesions at the branch surface. Then spots grow to about 1.5 cm and take on a dark reddish color (Fig. 2), and sometimes are bulky due to the fact that the injury induces the formation of gum below the affected epidermis. As the lesion progresses, cracks occur and the tissue begins to detach from the crust. Older lesions may be joined together and involve considerable damage to the branches.

Fruit symptoms

The lesions are rounded, 0.2 to 1.2 cm in diameter, often dark and cause depressions in the rind; when the fruit is green, spots are pale green in the center with a yellow halo; subsequently, as the fruit matures, the center of the lesion becomes dark brown (Fig. 3). Fruits affected by leprosis will prematurely ripen, causing its fall.

Leprosis lesions only affect the rind of the fruit but not the internal condition. In Brazil and other countries where the disease is reported, it is very common to see more than 30 lesions per fruit (Zúñiga and Ramirez, 2002). These symptoms are associated with premature fruit drop and significant production losses. The diseased fruits change color, fade and are susceptible to decay.

The CiLV-C virus has been successfully transmitted mechanically to the sweet orange and from the sweet orange to the herbaceous hosts *Chenopodium amaranticolor*, *C. quinoa* and *Gomphrena globosa,* used as indicator plants (Colariccio *et al*., 1995).

According to its morphology, CiLV has been considered part of the family Rhabdoviridae. However, Localli *et al*. (2005) suggest that the virus is an RNA with a bipartite genome, unlike the typical monopartite rhabdovirus genome. Recently, this information was confirmed by the complete CiLV-C genome sequence and phylogenetic analysis (Bastianel *et al*., 2006; Pascon *et al*., 2006). The complete CiLV-C nucleotide sequence confirms that the virus has a bipartite RNA; RNA 1 contains two open reading frames (ORFs) corresponding to 286 and 29 kDa, and RNA 2 contains four ORFs corresponding to 15, 61, 32 and 24 kDa. Phylogenetic analysissuggests that the citrus leprosis virus belongs to the Rhabdoviridae family and should be considered a type of the *Cilevirus* genus (Locali *et al*., 2006).

Locali *et al*. (2003) developed the first specific method for the molecular diagnosis of the disease through RT-PCR, which allows rapid and efficient virus detection. This technique has been useful in detecting CiLV-C in Brazil and other countries where the disease needs to be diagnosed for the first time (Freitas *et al*., 2003 and 2005, Gómez *et al*., 2005; Bastianel *et al*., 2006).

The detection of the virus in Colombia, by electron microscopy studies, was conducted by Kitajima, who confirms the presence of bacilliform particles contained in the endoplasmic reticulum of vascular parenchymal mesophyll cells, and an irregular shaped viroplasm in the cytoplasm. These observations ensure that the citrus leprosis virus in Colombia is a cytoplasmic type CiLV-C (León *et al*., 2006).

Molecular RT-PCR tests for the *Citrus leprosis virus* in Colombia initially showed positive results for some samples from Meta and Casanare using MPF (5'-CGTATTG-GCGTTGGATTTCTGAC-3') and MPR (5'-TGTATAC-CAAGCCGCCTGTGAACT-3') primers. In those studies, RNA fragments were amplified for samples collected in Meta, and one of the amplicons was sequenced and registered in the NCBI-GenBank as accession DQ272491. The obtained sequence showed 98% identity with the Brazilian nucleotide sequence isolated by CiLV-C from Brazil (NCBI-GenBank Accession AY289190.1), which allowed the first report of the occurrence of the CiLV-C in Colombia (León *et al*., 2006).

Afterward, Lenis and Angel (2010) tested, by RT-PCR, 15 different pairs of primers for CiLV-C detection, and found that only LCL1/MP4 was able to detect the virus in samples from Colombia, but results were not reproducible. This result confirms that leprosis virus detection in Colombia by RT-PCR techniques has no specificity for molecular amplification of different regions of the genome, suggesting that CiLV-C in Colombia shows differences from previous sequences reported in the NCBI-GenBank, so the virus should be sequenced to determine the variability among isolates.

Epidemiology

Symptoms of infection appear 15-60 d after mite transmission. Mite larvae transmit the disease with 48.3% efficiency but nymphs and adults are less efficient at transmission (Chiavegato, 1996). Only mites that have had access to lesions can transmit leprosis and an access period of 2 d is enough to acquire the virus. The virus is a lifetime-circulative type inside the mite body and transmission is possible once the mite acquires it (Chagas *et al*., 1983). According to recent studies, the virus is not systemic, which

FIGURE 1. Typical initial leprosis symptoms in orange leaves. Meta and Casanare, Colombia.

FIGURE 2. Symptoms of leprosis in Valencia orange branches. Meta and Casanare, Colombia. Left: initial state. Right: advanced.

means it does not spread through the plant (Kitajima *et al*., 2003). When the inoculum and virus vector are present in the field and there is no control, the entire cultivation could be contaminated in two or three years Rodrigues *et al*. (2001). Therefore, most leprosis control studies are focused on vector management (Alves *et al*., 2005). Lesions grow 5 to 7 mm in diameter every 15-20 d. When the severity is greater than 30%, affected leaf drop occurs on average 70 d after onset of the first lesion (León *et al*. 2006).

The Instituto Colombiano Colombiano (ICA) reports 3768 ha for citrus production in Meta on 1,084 farms, of which 48.5% were positive for leprosis. In Casanare, there are 529 ha planted with citrus on 275 farms, of which 52% were positive for the disease (Becerra *et al*., 2007).

FIGURE 3. Symptoms of leprosis in 'Valencia' orange fruit. Meta, Colombia. Left: initial state. Right: advanced symptoms.

The mite vector *B. phoenicis*

The red flat mite *B. phoenicis* is the main vector of the CiLV-C. The mite belongs to the Tenuipalpidae family and is recognized as the most damaging species in citrus-producing areas where the virus has been reported. It is a polyphagous specie, distributed in many tropical and subtropical regions of the world.

The *Brevipalpus* genus has more than 300 species, but only *B. phoenicis*, *B. obovatus* and *B. californicus* are registered as CiLV-C vectors. These three species have been reported in all continents; they are polyphagous and therefore are considered of great importance to agriculture (Carvalho, 2008; Chagas, 1983; Childers *et al*., 2003; Maia and Oliveira, 2002; Myers 2010). *B. phoenicis* is the main vector and is widely dispersed in the world. In the USA, *B. phoenicis* is present and distributed from south Florida to throughout the subtropical area (Childers *et al*., 2003).

In Colombia, the mite *B. phoenicis* is found throughout all regions of the country. It is recognized as the main vector of the citrus leprosis virus in the country and is included as a pest present in most citrus growing regions.

Description of *B. phoenicis*

The B. phoenicis mite passes through egg, larva, protonymph, deutonymph and adult stages. The larva, nymph and adult stages have dormant and feeding phases. The mite is able to reproduce asexually by parthenogenesis thelyotoky; sexual reproduction is less common (Chiavegato, 1996).

The *B. phoenicis* mite is about 0.3 mm long, with an oval, flattened and reddish body, with its front wider than the rear, with little spots that are clearer on the back of the body. They are located on the underside of leaves and fruits; in the nymph and adult stages, they have 8 legs but in the larval stage, they have 6 legs and (Fig. 4). Adults are 0.1 mm and their movements are slow. The eggs are rounded, reddish and individually placed in fruits or undersides of leaves and shoots (Chiavegato, 1996; León *et al*., 2006).

Life cycle of *B. phoenicis*

The longevity of the *Brevipalpus* species is two or three times longer than several Tetranychid mites. Under favorable environmental conditions (25°C; 65-70% RH), the complete cycle of *B. phoenicis* is 25 d (Haramoto, 1969). Each female lays one to four eggs per day for 20 d, which may produce several generations per year.

The *B. phoenicis* mite is associated with the *Elsinoë fawcetii* scab for protection against rain (Kitajima and Muller, 1972). When *B. phoenicis* colonizes affected fruits with scab lesions, the development is better than in healthy leaves and their immature stages are completed in less time; 14.4 d on fruits and 17.6 d on "Pera Rio" orange leaves; the fertility was higher in fruits than leaves with 39.2 and 8.6 eggs per female on average, respectively (Chiavegato, 1996).

The development of *B. phoenicis* in *Citrus lemon* is influenced by temperature and relative humidity. With 25 to 30°C temperatures and 70% relative humidity, the red flat mite's development time is shorter, with the longest oviposition period and better fecundity and egg viability (Sadana and Kumari, 1991).

The life cycle of *B. phoenicis* at 27.6±0.7°C and 69±7.9% relative humidity is: egg 4-6 d; larva 3-4 d; protonymph 5-7 d; deutonymph 6 to 8 d and adults 21 to 24 d. According to

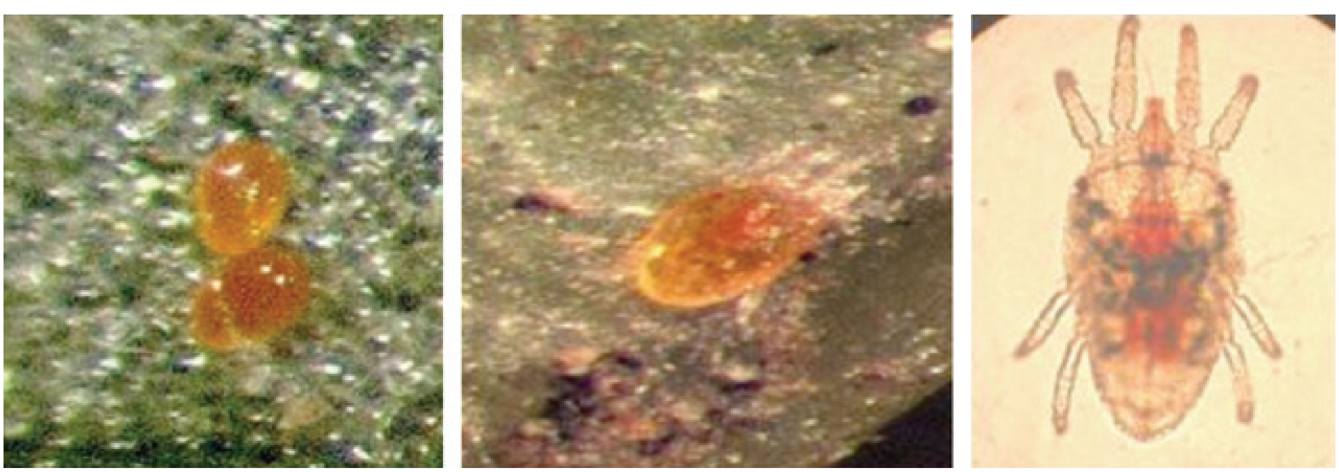

FIGURE 4. Development stages of red flat mite *B. phoenicis*. Left: eggs; center: nymph; right: adult.

these data, the life cycle from egg to adult is 18 to 25 d and the average adult stage is more than 21 d (León *et al.*, 2006).

Habits, hosts and characteristics of *B. phoenicis*

The red flat mite *B. phoenicis* injects toxins into fruits, leaves, branches and other tissues of many plants and its importance is highly associated with the ability to transmit the virus. The virus multiplies in the vector and once acquired, the mite can transmit it during its entire lifetime (Chagas *et al.*, 1983; Kitajima *et al.*, 2003).

B. phoenicis is able to live and multiply in several plants. Childers *et al.* (2003) reported 486 host plants besides citrus. Among cultivated plants, they mention cashew (*Anacardium occidentale*), mango (*Mangifera indica*), papaya (*Carica papaya*), cassava (*Manihot esculenta*), cotton (*Gossypium* sp.), guava (*Psidium guajava*), passion fruit (*Passiflora edulis*), coffee (*Coffea arabica*), cacao (*Theobroma cacao*), and grape (*Vitis vinifera*). In Brazilian citrus orchards, there are important weeds such as sabia (*Mimosa caesalpiniaefolia*), mallow (*Malvaviscus arboreus*), bala (*Sida cordifolia*), dayflower (*Commelina benghalensis*), goatweed (*Ageratum conyzoides*) and marigold spanish needle (*Bidens pilosa*) which are common hosts of the red flat mite (Maia and Oliveira, 2002).

Ulian and Oliveira (2002) found that *B. phoenicis* lives in citrus windbreaks and so recommended that *Hibiscus* sp. and *Bixa orellana* should not be used as living barriers because they favor survival of the mite.

León *et al.* (2006) recorded several weed hosts of the mite in citrus orchards such as verbena (*Stachytarpheta cayennensis*) yerbamora (*Lantana camara*) and escobo (*Sida* spp.). Natural infection of CiLV-C and mite presence in the *Swinglea glutinosa* plant, used in Colombia as a living fence, have also been reported (León *et al.*, 2008).

Population dynamics of the mite *B. phoenicis*

The dispersion of *B. phoenicis* is relatively limited; mites move less than 1 cm d^{-1}; with 3% reach distances of 40 to 50 cm; wind speeds of 30 to 40 km h^{-1} spread only less than 1% of the mite population located in fruits. The dispersion of *B. phoenicis* is very restricted compared to other species of citrus mites and this low dispersion requires considerable attention to the management of acaricide resistance (Alves *et al.*, 2005).

The *B. phoenicis* mite could be present in all aerial organs of orchards. The leaves are considered the prevalent reservoir, but the largest number of mites lives on the fruits. In southeastern Brazil, during the rainy season (October to March), the number of mites tends to decrease (Oliveira, 1996; Rodrigues *et al.*, 2001).

In Colombia, the *B. phoenicis* mite is able to infest citrus orchards throughout the year, with population increases when the rains fall and during the beginning of fruit growth. When precipitation increases, there are decreases in the mite population. Populations remain throughout the year and present slight increases during July, August and October in the Eastern Plains due to rainfall decreases. During these months, fruits are in formation and growth, which could encourage the spread of the disease (León *et al.*, 2006).

Control

According to Childers *et al.* (2003), when citrus leprosis is detected in a region, one has to prevent the spread to other areas free of the disease, while developing a control program. The program begins with the identification of the disease and the determination of the affected area. The subsequent steps are based on legislative actions and quarantine of the affected region. It also requires training in monitoring and controlling the mite vector and disease. To be successful, the program must achieve mite control and eradication of the disease in the affected region.

Rodrigues *et al.* (2001) emphasize that one orchard could be quickly infected when the virus inoculum is present in the field and there is no control of the mite vector. Therefore, control measures should be targeted to reduce the mite population and reduce the host plants of the virus. Chemical control of the mite should be performed technically, based on mite monitoring and using different chemical groups of acaricides to avoid resistance.

Focus control of *B. phoenicis* is also a strategy for management of CiLV-C because it is an economical and efficient strategy of vector control; and reduces the regular use of chemicals and the possibility of resistance development (Alves *et al.*, 2005). Other factors to be considered in an integrated control of the leprosis virus - vector program are discussed below:

Chemical control of *B. phoenicis*

The costs of acaricides for control of the CiLV-C vector *B. phoenicis* in orange plantations of Brazil are about $100 million dollars and this represents about 25% of total production costs. In Sao Paulo in 1995, over 60% of citrus orchards had leprosis, which explains why chemical vector control is widely used (Rodrigues *et al*., 2001). After achieving an effective control of the mite, it takes two to three years to fully recover for a severely affected tree (Ferreira *et al*., 2003; Rodrigues *et al*., 2003).

Childers (1990) found that agricultural oils in combination with pyridaben, fenbutatin-oxide, dicofol or high doses of sulfur give mite control of 35 d; ethion carbaryl is less effective and did not control *B. phoenicis*.

There are several reports of pest resistance to organoestanic acaricides (cyhexatin, fenbutatin oxide, azocyclotin) in Brazil. Mite resistance has already been detected and characterized to dicofol, propargite and hexythiazos acaricides (Omoto *et al*., 2000). In addition, reduced mite susceptibility was found to enxofre pyrethroid acaricides, lime sulfur, as well as abamectin. In Brazil, the intense use of abamectin and enxofre for citrus rust mite control has already ended in critical points of resistance levels. The management strategy of pest agrochemical resistance is therefore essential in managing the leprosis mite (Omoto and Alves, 2004).

To determine the control of *B. phoenicis,* Corpoica tested 12 different acaricides, acrinathrin, tetradifon, sulfur, propargite, clofentezine, abamectin, tetradifon / propagite, milbecmectin, mineral oil, fenpyroximate, clorfenapyr and amitraz; all products result in satisfactory control. Seven products give a control above 80% and only five of the 12 treatments (abamectin, amitraz, acrinathrin, sulfur and tetradifon) show efficacy below 80% after 15 to 30 d of application. Therefore, in Colombia, there is an availability of specific acaricides to control the leprosis mite *B. phoenicis* with over 80% efficiency (León *et al*., 2006).

Biological control

Among the natural enemies of the *B. phoenicis* mite are the predatory mites of the Phytoseidae family and the entomopathogenic fungus *Hirsutella thompsonii*. Carvalho *et al*. (2008) studied the population dynamics of *B. phoenicis* and predatory mites (Phytoseidae and Stigmaeidae) in coffee plantations in Sao Paulo, Brazil; they found higher mites populations of *B. phoenicis* during dry periods of the year. The predatory mites *Euseius citrifolius* and *E. concordis* were the most frequent.

Biological alternatives for control of the citrus leprosis virus are a good choice for part of the integrated control of *B. phoenicis*. Predatory mites are considered the most efficient natural enemies of phytophagous mites. Citrus mite predators are frequently found in Colombia; mites of the Phytoseidae family such as *Euseius* sp., *Iphiseiodes zuluagai*, *Amblyseius* sp. and *Phytoseiulus* spp. are the most important natural enemies of the mite vector in citrus orchards (León, 2001).

Alternatively, control includes the use of entomopathogenic fungi; *H. thompsonii* and *Metarhizium anisopliae* var. *acridum* have been recorded as very promising natural enemies for *B. phoenicis* mite control (Magalhaes and Rodriguez, 2005). Rossi *et al*. (2006) recorded *H. thompsonii* as the most virulent entomopathogenic for red flat mite control.

Other control alternatives

Recently, it was shown that a bacterial endosymbiont of the genus *Cardinium* blocks production of androgenic hormones in the early stages of mite development, resulting in feminization of haploids males (Novelli *et al*., 2004). As a consequence, it is commonly found that less than 1% of the population is male. Cobalt 60 irradiation to treat the bacteria *Cardinium* sp. influences the oviposition period and the number of eggs laid by irradiated females (Novelli *et al*., 2008).

Tangor Murcot is the most Tangor variety produced in Brazil, it can host the CiLV-C and be asymptomatic (Bastianel *et al*., 2004). Genetic improvement shows that the segregation of the disease phenotype in a population obtained by crossing the F1 sweet orange variety Pera and Tangor Murcot, pathogen susceptible and resistant respectively, suggests that some genes are involved in resistance to the citrus leprosis virus (Salvo, 1977; Bastianel *et al*., 2006).

Similarly, the progress achieved with the molecular genome decoding of the CiLV-C virus may result in the creation of transgenic plants with virus resistance. Researchers believe that the use of these transgenic plants in the near future will result in the suspension of acaricide applicationss to control the vector of the leprosis virus (Pascon *et al*., 2006).

Conclusions and recommendations

The citrus leprosis virus CiLV-C is an economic and important disease representing losses for the citrus industry in the countries where it has been established.

The disease was first observed in Colombia during 2003, in the Eastern Plains region. After detection of the disease, a phytosanitary alert and prevention campaign, quarantines and research plans looking for the solution to the problem were launched. Currently, due to the presence of the disease in Colombia, one should expect major restrictions for the international marketing of citrus and other agricultural exports.

Given that the virus diagnostic of leprosis citrus in Colombia with the RT-PCR molecular technique has not been reproducible, unspecific amplifications and therefore detection are unreliable; it is suggested that the CiLV-C virus in Colombia should be sequenced again to determine the variability of genomic isolates registered in the NCBI-GenBank; and subsequently, the RT-PCR molecular test has to be standardize with the new genomic isolates that are obtained.

Even though methods and technologies for the control of the mite vector and the citrus leprosis virus in Colombia are available, in order to eradicate the disease from the country, control programs require further investigation on the aspects of the mite vector and virus-plant relationship; and also on the biological control of the vector and genetic management of the disease to determine the most effective control methods applicable to the circumstances of the country.

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