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Citrus Leprosis

Centennial of an Unusual Mite–Virus Pathosystem

Citrus leprosis is one of the most economically important diseases of the Brazilian citrus industry. It is characterized by the presence of local lesions on fruits, leaves, and twigs or branches, which directly reduce production and lifespan of citrus plants. The etiologic agent is the Citrus leprosis virus C (CiLV-C) transmitted by tenuipalpid mites of the genus *Brevipalpus*. Every year, control of the vector mite costs Brazilian growers around US\$80 million. This disease was originally described from the State of Florida in the United States over a hundred years ago, and was found later in Paraguay, Uruguay, Argentina, and Brazil, where it is endemic. Recently, CiLV-C was detected in Bolivia, Venezuela, and Colombia, and it is spreading northward through the countries of Panama, Costa Rica, Nicaragua, Guatemala, Honduras, El Salvador, and Mexico. This proximity and the potential damage that it can cause concerns the United States, where the disease has not been observed since the 1960s, and the Caribbean Islands, which are still leprosis-free. Until recently, one of the problems for citrus leprosis management was diagnosis, which has been based primarily on symptoms. That could be misleading, because other pests and pathogens may cause lesions similar to those associated with leprosis. For the same reason, reports of the disease in countries in Asia and Africa must be reviewed. If the presence of CiLV-C on the Asian and African continents is not confirmed, then citrus leprosis has been limited to the American continent. The use of electron microscopy and, more recently, the development of molecular tools increased the precision and reliability of CiLV-C detection. Hence, many of the old reports of citrus leprosis in different areas of Brazil could be confirmed, expanding the known area of occurrence. Today, citrus leprosis is confirmed throughout Brazil, indicating the need for management in commercial orchards. Other countries where the virus is also confirmed need management strategies as well. Where the disease is not yet reported, there is a need to avoid its introduction. In this review, historical details and new information on citrus leprosis dispersion and its geographic distribution are brought together from numerous reports.

First Reports

Citrus leprosis was first reported from Florida in the beginning of the twentieth century, where it was initially called scaly bark because of the characteristic stem lesions and bark scaling symptoms. The disease was also named nail head rust because of the typical lesions on the fruits and stems (Fawcett, 1907; 1911 cf. Rodrigues et al. [81]). In 1926, the disease was called leprosis by Fawcett and Lee (8). In South America, citrus leprosis was reported for the first time from Paraguay by Spegazzini (91), and it was initially named “lepra explosiva”. Around 1930, “lepra explosiva” was considered to be the same disease reported from Florida (29). Soon after the report from Paraguay, leprosis was observed in Argentina and Uruguay (7). Bitancourt (8) cited in his review that there was an interception of suspect material from Bolivia by the U.S. quarantine service; however, the official report of the presence of leprosis in that country was made decades later (37).

There are old reports, from the beginning of the twentieth century, of the occurrence of citrus leprosis in the south of China, north of India, Ceylon, Japan, the Philippines, Java, Egypt, and South Africa (8,21,29). However, further reports of the occurrence of this disease in these countries could not be found. In a comprehensive review of plant viruses from Asia, Murayama et al. (63) do not mention the presence of citrus leprosis. There is one unique report of leprosis in India, dated 1978 (21). It is important to note, however, that this report was based only on symptoms. If the original reports were correct, the disease might have disappeared from these places, as it probably did from Florida. It is suspected that, in Florida, adverse weather conditions associated with intensive sulfur application on citrus orchards drastically reduced the vector population and completely eliminated leprosis inoculum (20). Citrus leprosis might still occur in some of the previously reported regions, but with low economic importance through the years. Characteristic symptoms and association with the mite vector are still good indications of the presence of leprosis in a particular area; however, confirmation of the disease is obtained by cytopathology and molecular detection of the virus (4).

In Brazil, citrus leprosis has been reported since the 1930s. It was originally found in Navel sweet orange (*Citrus sinensis* (L.) Osbeck) in 1931 in Sorocaba, São Paulo (6). The abundance of leaf symptoms observed in Brazil (contrary to what happened in Florida, where leprosis leaf symptoms were very rare) delayed identification of the disease, which was then called “variola”. Two years later, it was concluded that this disease was the same one

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known as leprosis in Florida (8). Even with the variation in symptoms, Bitancourt (8) reported the disease in other citrus species such as sweet lime (*C. limettioides* Tan.), Key lime (*C. aurantifolia* (Christm.) Swingle), Rangpur lime (*C. limonia* Osbeck), sour orange (*C. aurantium* L.), citron (*C. medica* L.), grapefruit (*C. paradisi* Macfad.), and mandarin (*C. reticulata* Blanco). Recently, many of these species were considered resistant or asymptomatic to leprosis (4,32).

The Citrus Leprosis Pathosystem

The etiology of citrus leprosis was initially contested. In the United States, Fawcett and Burger in 1911 (cited by Bitancourt [8]) suggested that the disease was caused by the fungus *Cladosporium herbarium* var. *citriculm* Faw. Even before that, other fungi such as *Hysterographium*, *Colletotrichum gloeosporioides*, and *Hormodendron*, cited by Fawcett, were associated with scaly bark (cf. Rodrigues et al. [81]). In Paraguay, Spegazzini (91) suggested that the causal agent of “lepra explosiva” was the fungus *Amyliroa aurantiorum* Speg. Because the disease arose in Brazil, even without the etiological agent identified, Bitancourt (8) believed that leprosis was caused by a virus, because of the symptom similarity with ring spots caused by this type of pathogen.

In Argentina, Frezzi (36) and Vergani (94) demonstrated that citrus leprosis was transmitted by the mite *Tenuipalpus pseudocuneatus* Blanch, later identified as *Brevipalpus obovatus* Donnadieu. The association of mites with the disease was confirmed in the United States, and *B. californicus* Banks was identified as the vector in Florida (52,53) and in Guatemala (75). In Brazil, Musumeci and Rossetti (64) reported the presence of *B. phoenicis* Geijskes in plants with leprosis symptoms.

With the association of *Brevipalpus* spp. and citrus leprosis, the symptoms were attributed either to toxins from the mite’s saliva (94) or to a pathogen, probably a virus, transmitted by the mite (36). In Florida, Knorr (53) demonstrated that leprosis could be transmitted through the insertion of symptomatic shoots by tip graft to healthy plants. Graft transmission of leprosis was confirmed in Brazil by Chagas and Rossetti (14). Kitajima et al. (50) observed the presence of bacilliform particles associated with leprosis lesions by transmission electron microscopy (TEM), reinforcing the hypothesis that a virus was the causal agent. Finally, Colariccio et al. (22) obtained typical symptoms of leprosis by mechanical sap inoculation in plants of the same genus and other herbaceous plants (*Chenopodium amaranticolor* Coste & Reyn., *C. quinoa* Willd., and *Gomphrena globosa* L.), proving the viral cause of leprosis.

Leprosis involves symptoms in citrus leaves, stems, and fruits, which may vary according to the host species, the stage of development of the plant, and the pathogen isolates (85) (Fig. 1). Typical lesions are chlorotic or necrotic, varying in color from light yellow to dark brown, and are often circular with diameter ranging from 5 to 12 mm, localized only on the feeding sites of the mite vector. Sometimes it is possible to observe a darker central point in older lesions, and ring spots may also occur. Particularly when manifested under high temperature, the center of the necrotic leaf tissue may tear apart. These symptoms exhibit a common pattern but extremely variable among citrus species and varieties (4). The disease can cause a decrease in production due to reduction in tree canopy development, premature fruit and leaf drop, and dieback. Under high inoculum conditions, it can cause the death of young susceptible plants (80).

The occurrence of virus-like particles in symptomatic leaf tissues was reported by Kitajima et al. (50), who did not find such particles in surrounding, asymptomatic areas. In symptomatic leaf tissues, bacilliform particles were found in the nuclei of infected cells and caused cytopathological effects similar to those induced by Orchid fleck virus (OFV). Later, electron dense viroplasm and shorter bacilliform particles were observed in the endoplasmic reticulum of symptomatic tissue (22,51). This virus has been referred to as Citrus leprosis virus, cytoplasmic type (CiLV-C) (Fig. 2A), which is the prevalent form, in opposition to the one

described in 1972, referred to as Citrus leprosis virus, nuclear type (CiLV-N) (81) (Fig. 2B). Recently, CiLV-C is under consideration for status as full species designated Citrus leprosis virus C by the International Committee on Taxonomy of Viruses (ICTV) (44). Because of this new nomenclature, we suggest that the CiLV-N should be named Citrus leprosis virus N. The CiLV-N occurs rarely, and is reported only in Boquete, Panama (26) and in some cool-weather localities in the states of São Paulo (Amparo, Monte Alegre do Sul, and São Roque), Rio Grande do Sul (Marques de Souza), and Minas Gerais (Andradas) in Brazil (13,33,48). Lesions

Fig. 1. A to C, Typical leprosis symptoms in citrus leaves, fruits, and stems caused by Citrus leprosis virus C (CiLV-C). D, Uncommon CiLV-C-induced symptoms in fruits.

Fig. 2. Micrographs of A, Citrus leprosis virus C (CiLV-C) and B, CiLV-N particles transmitted by C, *Brevipalpus phoenicis*.

Fig. 3. Symptoms of leprosis caused by Citrus leprosis virus N (CiLV-N) in citrus leaves.

caused by CiLV-N resemble those caused by CiLV-C, but tend to be smaller with a necrotic center and a chlorotic halo (Fig. 3). Ring spots, usually associated with leprosis caused by CiLV-C, are not observed in CiLV-N-infected plants (4). There is very little information available on CiLV-N, but since it does not share genomic sequences with CiLV-C, they are considered to be different viruses (34).

Leprosis is indeed an unusual disease. It can be caused by two completely distinct viruses with similar morphology and vector.

Both CiLV-C and CiLV-N are bacilliform and transmitted by tenuipalpid mites of the *Brevipalpus* genus (Fig. 2C). Few other plant viruses are transmitted by mites, and most of them are from the eriophyid family. In addition, the virus does not spread systemically even in its primary host, and causes only localized lesions where particles accumulate.

These data clearly show that this is not a typical plant virus. In fact, the sequencing of the CiLV-C confirmed that it has a ss+ RNA bipartite genome, with RNA1 of 8,745 nucleotides (nt) and RNA2 of 4,986 nt. The RNA1 contains two open reading frames (ORFs) corresponding to a 286-kDa protein with four domains putatively involved in replication, and a 29-kDa protein that is possibly the coat protein. The RNA2 contains four ORFs that correspond to 15-, 61-, 32-, and 24-kDa proteins, respectively. Only the 32-kDa protein has similarity with other known viral proteins, and is possibly involved in cell-to-cell movement of the virus (60). Contrary to what was assumed earlier, the virus does not belong to the *Rhabdoviridae* family (60,75), and according to Locali-Fabris et al. (60), it should be classified as the type member of a new virus genus denominated Cilevirus. This classification has been approved by the executive committee of the ICTV and is awaiting ratification (44). On the other hand, the genome of OFV, another virus transmitted by *Brevipalpus* mites that accumulates in the nucleus of infected cells and is considered a nuclear type of the *Brevipalpus*-transmitted viruses (BrTVs) was completely sequenced and revealed genomic sequence and organization similarities with members of the *Rhabdoviridae*. However, because of its bipartite genome, OFV was tentatively classified as the prototype of a new genus of plant viruses, *Dichorhabdovirus* (54). Because CiLV-N is also a BrTV that accumulates in the nucleus of infected plants and has similar morphology to OFV, it is possible that it will be classified as a species of this new genus in the future. However, to date there are no ongoing studies on this issue.

It is now known that the CiLV-C host range is significantly wider than originally supposed. Recent experiments showed transmission by viruliferous mites to *Solanum violaeifolium* (82), *Phaseolus vulgaris* (39), and to several other species of the vegetation found in hedgerows and windbreaks that occur near citrus orchards (70). Additionally, the first noncitrus natural host of CiLV-C was found, the rutaceous plant *Swinglea glutinosa*, often grown in hedgerows in Colombia (55). However, at this point it is not known whether or not the alternative hosts of CiLV-C play any role in the disease epidemiology, but this subject is being addressed by our group due to the potential impact on field leprosis management.

All active stages of the *Brevipalpus* mite (larvae, protonymph, deutonymph, and adult) can acquire and inoculate the virus, which is not transovarially transmitted (15,67). An old, unconfirmed report of transovarial transmission of CiLV by *B. obovatus* (53), associated with the detection of viral particles similar to CiLV within the bodies of viruliferous *B. phoenicis* (83), led to the hypothesis that the virus could replicate in the vector (80). How-

ever, recent studies with better preserved material did not confirm this observation, and indeed, it is suspected that the particles described were the result of fixation artifacts. On the other hand, Kitajima et al. (49), working with material properly preserved and from different origins, found presumed virions between the membranes of epithelial cells of intestinal diverticulum and adjacent tissue of *B. phoenicis* that had access to leprosis lesions. Confirmation that those were indeed CiLV-C particles came from immunolocalization studies with antiserum produced against the putative capsid protein of the virus (46,61). However, the same method did not detect in *B. phoenicis* the presence of viroplasm-like the ones found in the cytoplasm of infected plants (46,47). These data, together with data on viral titers obtained by real-time PCR analyses (65,66), indicate that CiLV-C circulates, but does not replicate, in the mite vector, weakening the hypothesis that it might be a mite virus transitioning between hosts in different taxonomic kingdoms.

Another outcome from this new information relates to the management strategy for leprosis. Currently, growers spray acaricides after an empiric threshold (often incidence of 10% of mites found in assessed fruits and branches) is reached. Field scouting is typically done every other week, with 1 to 2% of the trees inspected. Because mite population density is normally low and their distribution in the orchard is uneven, sampling is not accurate (3). In addition, the economic and environmental impacts of the use of large amounts of chemicals are high and, to a certain extent, unnecessary. This is because the threshold takes into consideration the incidence of mites in the orchard, regardless of the presence of the virus in the vector. Recent studies (3) showed low correlation between occurrence of mites and disease foci in the field, because only part of the mite population carries the virus. These data, together with the information that the virus does not replicate in its vector, clearly show that management recommendations for leprosis should be reviewed to include alternative measures to reduce the source of inoculum and mite population, such as pruning, and also to establish protocols to identify viruliferous mites in the field.

Leprosis Distribution in Brazil

Even though the worldwide distribution of leprosis has increased recently, Brazil is by far the country where it is more important and causes more damage as the most important viral disease of citrus. This is due to an array of factors that include environmental conditions that favor the vector, which colonizes citrus throughout the year, large contiguous areas planted with highly susceptible sweet orange varieties, and the endemic presence of the virus in traditional citrus growing regions. Additionally, epidemics have been reported in years with extensive water deficit periods, which favor mite reproduction and CiLV-C spread (73), and in years when citrus prices are low, which discourages growers from spraying with acaricides (81).

Leprosis is reported in each of the five Brazilian macro-regions where citrus is grown (Fig. 4). Even though it has occurred in Brazil for almost 80 years (5,8), leprosis is still one of the main problems of the São Paulo citrus industry (3), which is responsible for approximately 80% of Brazilian production (45).

In São Paulo, citrus leprosis is widespread (4,87), but its incidence and severity are higher in the traditional citrus belt of the center-north and northwest regions (2). In the south and southeast of the state, in newer citrus growing areas, the *Brevipalpus* mite is present, but leprosis incidence is still low (D. Joaquim, GTACC, *personal communication*). In the 1930s, in a laborious survey of leprosis in Brazil and neighboring countries, Bitancourt (7,8) observed symptoms of the disease in many orchards along the Uruguay River in Rio Grande do Sul State, in the state of Mato Grosso, and in the municipalities of Belém (Pará State), Ouro Preto, Lavras, Volta Grande, and Itajubá (Minas Gerais State), Uruçui (Piauí State), Jacarepaguá (Rio de Janeiro State), and Crato (Ceará State).

Leprosis is found in the four states of the southeast region of Brazil. Some isolates from Rio de Janeiro seem to differ biolog-

ically with respect to symptom severity in herbaceous hosts (23). The main production area in Minas Gerais State is Triângulo Mineiro, near the north of the São Paulo citrus belt. And even though leprosis is important mainly in that region of the state, the presence of CiLV-C was verified in other municipalities as well. In Minas Gerais, the citrus leprosis of the nuclear type was found in a municipality in the south of the state called Andradas (13). In Espírito Santo, the disease is also present (57), and occurs at high frequency in the whole state (H. Costa, Incaper, *personal communication*) in citrus orchards that are expanding in the region.

In southern Brazil, the oldest reports of the disease are from Rio Grande do Sul (7), but currently leprosis is also found in the two other states of the region, Paraná and Santa Catarina (Fig. 4). In this region, which is the main Brazilian producer of fresh fruit, the disease was already reported in many localities (16,48,58), but without significant yield loss. In Rio Grande do Sul, among several sampled places, an isolate of CiLV-N was found (33) in Navel sweet orange plants in a backyard planting. Since the presence of *B. phoenicis* in citrus orchards from the southern states is endemic (17,42,43), probably the disease is also endemic, but occurs in low incidence.

There are old reports of leprosis in the north region of Brazil (Fig. 4). According to Bitancourt (8), the disease was observed in Belém do Pará in the beginning of the 1940s. Recently, Boari et al. (10) confirmed the occurrence of leprosis in the municipality of Capitão Poço, 210 km east of Belém, through RT-PCR and TEM. In states like Amazonas, with a rising citrus industry, the first formal report of the disease was in 2006 (35), even though some growers maintain that they have seen the symptoms in the region for several years. In general, leprosis does not cause great damage to citrus production in this state, probably due to the wide variety of natural enemies of the vector (11,12,72). This is because citrus is often not grown in extended areas as a monoculture, which seems to promote a balance of microfauna including pests and their natural predators.

Fig. 4. Brazilian macro-regions. North: AC, Acre; AM, Amazonas; AP, Amapá; PA, Pará; RO, Rondônia; RR, Roraima; TO, Tocantins. Northeast: AL, Alagoas; BA, Bahia; CE, Ceará; MA, Maranhão; PB, Paraíba; PE, Pernambuco; PI, Piauí; RN, Rio Grande do Norte; SE, Sergipe. Midwest: DF, Distrito Federal; GO, Goiás; MS, Mato Grosso do Sul; MT, Mato Grosso. Southeast: ES, Espírito Santo; RJ, Rio de Janeiro; SP, São Paulo. South: PR, Paraná; RS, Rio Grande do Sul; SC, Santa Catarina. In gray, Brazilian states with reports of citrus leprosis. The disease is also present in the State of Roraima (RR), but there is no formal report on that occurrence.

Recent reports of citrus leprosis in the north region of Brazil were also made in the states of Tocantins (25) and Acre (31). In Acre, even though citriculture is characterized by small orchards, the disease can cause severe damage with the destruction of plants severely infected by the virus (G. W. Muller, *personal communication*). Leprosis was also identified in at least two municipalities of the state of Roraima: Boa Vista and Alto Alegre (41). Although there are no formal reports of leprosis in other states of this region, the virus may be present.

Leprosis also occurs in the Brazilian midwestern and northeastern regions (Fig. 4). Although the first reports in Mato Grosso and Ceará are dated from the 1940s (7), it was not until 2006 that leprosis was confirmed in Mato Grosso do Sul, in the municipality of Terenos (69). In Goiás, leprosis was reported as a concern to the citrus industry in the 1980s due to the high incidence of the vector *B. phoenicis* (93). Recently, the disease was confirmed in sweet orange and mandarin by RT-PCR in Goiânia and Cromínia municipalities (69). In the south of Goiás State, the disease occurs mostly in orchards that are not well managed (J. Meneguci, Embrapa Transferência de Tecnologia, *personal communication*). Similarly to what happens in the north of the country, leprosis should not cause significant damage to the new but fast-growing midwestern citrus industry. In Distrito Federal, where citriculture occurs on a small scale, leprosis has been known since the 1980s in some orchards and in Embrapa Cerrados experimental fields (E. W. Kitajima and N. T. V. Junqueira, *unpublished data*), although there is no formal report.

Bahia and Sergipe, localized in the northeastern region of Brazil (Fig. 4), are the second and third main citrus producing states, with 4.7 and 3.1% of the national production, respectively (45,77). Leprosis reports are recent in both states, and there is increasing concern because the vector's population density is high in the region (74) and sweet orange 'Pêra,' highly susceptible, represents up to 70% of the citrus cultivated in the area (78). Together, Bahia

and Sergipe represent 95% of the northeastern citrus production (78), and leprosis has already caused significant damage, mainly in Bahia, where the government encouraged eradication of infected trees in new areas and chemical control of the disease for several years after the first symptoms were observed in the state, in 1999 (89). Citrus leprosis was reported for the first time in Bahia, in the municipalities of Itapicuru and Rio Real on the north coast of the state that produces more than 50% of the citrus in the state (77). The disease is not present in the Recôncavo Sul region, which, with the northern coast, represents more than 80% of the citrus production of the state (77).

The citriculture of Sergipe is geographically close to the major producing municipalities of Bahia. Even though the growers and technicians of Sergipe Agricultural Bureau made a considerable effort to eliminate the disease foci in neighbor municipalities to avoid leprosis in the state, the disease was reported by Boari et al. (9). The symptoms of leprosis observed in this region, like those in Rio Real (BA), are quite different from those found in other regions of the country, exhibiting larger green ring spots (9; Fig. 1). However, a molecular diagnostic tool used for the detection of CiLV-C in samples from different regions of Brazil and abroad (34,59) detected the virus in samples with atypical symptoms as well, proving that they are caused by the same pathogen (9).

To date, there are no formal reports of leprosis in other states of the Brazilian northeast; however, a survey is necessary to confirm that they are leprosis-free regions. With the citrus industry advancing to new areas, it is possible that the disease distribution will increase once the vector is found countrywide.

Leprosis Worldwide Distribution

In 1999, leprosis was reported in Venezuela (79), and by 2000 it had reached Panama and Costa Rica in Central America (1,26). It is currently reported in several South and Central American countries, and in Mexico in North America (4,81). Recent formal reports of leprosis occurrence were based on characteristic symptoms, TEM analyses, and in some instances, the RT-PCR method developed by Locali et al. (59). The disease is now also confirmed in Guatemala (62), Bolivia (37), Colombia (56), and Honduras (84). Additionally, according to OIRSA (Organismo Internacional Regional de Sanidad Agropecuaria), which includes Mexico, Belize, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, and Panama, in 2003, typical symptoms of citrus leprosis were found in every Central American country except Belize (71) (Fig. 5). Recent surveys in Belizean citrus orchards supported the leprosis-free status of the country (S. Williams, Citrus Research & Educational Institute of the Belize Citrus Growers Association, *personal communication*).

The recent northward spread of the disease within the Americas is a fact, but there are several questions regarding how this is occurring. Leprosis virus is found localized only in the conspicuous lesions; hence, contrary to other citrus viruses and viroids that infect their hosts systemically, transmission by infected budwood is very unlikely. Experimental transmission of CiLV by budwood is possible but difficult to achieve (14,53,94). It is possible that plants infected by the virus or carrying viruliferous mites were taken to new citrus growing areas. It should be noted that total production and consumption of citrus has increased significantly since the 1980s, with oranges, which are highly susceptible to leprosis, accounting for more than half of global citrus production in 2004 (92). Additionally, the mite has the capability of dispersion by itself and also by the wind; hence, viruliferous mites may have arrived in new areas and been responsible for the introduction of the disease.

The most likely hypothesis, though, is that the disease has occurred unnoticed in some of these areas for long periods of time. The symptoms of leprosis are typical, but sometimes it takes years for the growers to actually observe them or acknowledge the problem in their orchard. In São Paulo State, Brazil, when the inoculum is present in an area and acaricides are not applied, the disease typically spreads through the orchard and reaches levels

Fig. 5. American countries with reports of citrus leprosis.

causing perceptible yield damage in 2 to 3 years. This is because disease incidence and severity progress rates are not as high as those for other citrus diseases; however, because leprosis is a polyetic disease, the amount of infected tissue, as well as initial inoculum, increase yearly (24).

Information obtained from growers supports this idea. In Guatemala, growers mentioned that they saw symptoms of leprosis in their orchards years before the identification of the causal agent. In some cases, the symptoms were confused with those caused by psoriasis, particularly when branches were affected. In other cases, the growers simply did not know what caused the symptoms, but since they were not widespread within the orchards, producers were not concerned about them. Only after the buildup of inoculum and greater incidence and severity of disease in the field did the growers seek help. This scenario may have contributed to the perception that the disease remained limited to an area for years and suddenly spread to new areas.

The official report about citrus leprosis from OIRSA brings interesting details about the geographic localization of disease foci in the member countries and mentions that the incidence of leprosis is especially severe in Guatemala, where the disease seems to have occurred since 1995 despite its recent report (71,75). In this country, the disease is forcing growers to replace sweet orange groves with other citrus species that show some tolerance or resistance (71). According to OIRSA (71), El Salvador also showed severe and apparently old leprosis foci, with original inoculum probably introduced from Guatemala.

The recent establishment of citrus leprosis in Central America represents a potential threat to citriculture in North America, the second most important citrus producer in the world, and to other citrus producing countries. It could be said that there is an imminent threat of the reintroduction of the disease into the United States (18) and the Caribbean Islands within a few years. Also, due to disease dissemination in new American countries, its importance has increased significantly in the latest years, such that it is now included in the quarantine list of important pests in Europe (28). In Colombia, even after preventive measures were adopted, virus presence was confirmed in a few years (56). In this country, citrus leprosis occurs only in the Llanos Orientales region (Departments of Meta and Casanare). For that reason, CORPOICA (Corporación Colombiana de Investigación Agropecuaria) is distributing information about leprosis and trying to avoid its dissemination to leprosis-free regions. However, researchers from ICA (Instituto Colombiano Agropecuario) have recently found leprosis in Ibagué, Department of Tolima, showing that the disease has reached the mountains (S. E. A. Díaz, L. A. Castañeda, and J. Freitas-Astúa, *unpublished data*).

Many countries or regions tried to eradicate citrus leprosis after its first report, but frequently they began too late, simply because when the symptoms are identified, the disease has already spread in the region for some time due to its slow dissemination rate. A classic example of success in disease eradication is the United States. The first country to describe citrus leprosis, it currently has *Brevipalpus* spp. but is free of the disease. Leprosis was eradicated from the country probably due to a combination of applications of sulfur acaricides and unfavorable climatic conditions such as frosts, which together were able to eliminate the viruliferous mites and the inoculum from infected trees at least 30 or 40 years ago (19). Additionally, it is possible that the virus present in Florida was the CiLV-N. This hypothesis was raised based on the symptoms reported, and on the facts that CiLV-N tends to be found in cooler regions and is less efficiently transmitted by *Brevipalpus* spp. If this hypothesis is true, the relatively low fitness of the virus could have contributed to the success of leprosis eradication in the United States. Recent efforts to eradicate leprosis were made in Costa Rica and Mexico. In 2000, the disease was identified in Costa Rica near the Panama border—a region of forests with little or no tradition of citrus cultivation. The few symptomatic plants were eliminated and the country was considered free of the disease (L. Moreira, Universidad de Costa Rica, *personal communication*).

However, with the identification of leprosis in Nicaragua (27,40), on the northern border of Costa Rica, the disease is threatening Costa Rica again, and this time near the citrus producing area of the country. In Mexico, soon after the leprosis outbreak in Chiapas in 2005 (88), an emergency program for its eradication was established. In 2006, the “Norma Oficial de Emergencia” (NOM-EM-046-FITO-2006) was published, in which an Emergency Official Standard was created with the objective of confining, eradicating, and preventing leprosis dissemination in Chiapas State (86). Even with the efforts to contain the disease, it was recently observed in 2007 in the State of Tabasco (90). Although it is still restricted to the south of Mexico, there was an apparent advance of the disease. In other countries, such as Guatemala and Colombia, where leprosis identification was made when the disease had already spread into several orchards, the producers are living with the disease (56,71).

Final Considerations

Citrus is grown in 22 of 27 Brazilian states, but about 99% of the sweet oranges produced originate from nine states: São Paulo, Bahia, Sergipe, Minas Gerais, Paraná, Rio Grande do Sul, Santa Catarina, Goiás, and Rio de Janeiro, situated in the southeast, northeast, south, and midwest regions (45). In all of them, citrus leprosis is present, although it assumes greater economic importance in São Paulo State, which is responsible for more than 80% of Brazilian sweet orange production.

In almost all of the American countries where leprosis was reported in the beginning of the twentieth century, the disease is still present, but its importance had been restricted to Brazil, especially São Paulo State, during most of those years. However, after the recent reports in new areas, the relevance of the disease and the losses it causes have significantly increased. Old reports of the disease on other continents are likely misinterpretation of symptoms and need further confirmation.

Although leprosis is a disease known for more than one century, its current importance is undeniable. For years, researchers addressed mainly aspects related to vector biology and control and, in a few instances, studies on responses of some citrus species and varieties to the virus. While the results were important and informative to citrus growers, they dealt with only part of the problem. In recent years, research has been focused on the pathosystem. Examples include the epidemiological studies conducted by Bassanezi and Laranjeira (3) and Czermainski et al. (24), and studies on the inheritance of resistance against the virus (5), developing a quick and efficient method of diagnosis (5), the possibility of tracking viruliferous mites in the field (67), the CiLV-C genome (60,76), identification of differentially expressed genes in symptomatic plants (30), the presence and role played by endosymbionts in the vector (38,68,95), and the investigation of virus–vector interactions (65,80). These studies have already brought new insights about this complex pathosystem, contributing to a more efficient management of the disease, and benefiting growers and consumers of our daily orange juice.

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