Plant Science Horizons

Review

Open-Access

Plant Science Horizons 2026, 1(1): e0006
https://doi.org/10.66835/psh.2026.e0006

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Phloem Limited Citrus Pathogens in The 21st Century: Distribution, Diagnostics, and Management Strategies

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Abstract

Citrus is regarded as a fruit crop that is economically significant worldwide, serving as a source of nutrition, a driver of job creation, and a key contributor to global agricultural trade. However, the agricultural productivity of citrus is being limited by a set of devastating diseases that are caused by the phloem-affirming or phloem-confined pathogens commonly known as the plasma diseases. Diverse biological agents such as phloem-limited bacteria, including Candidatus Liberibacter spp. Spiroplasma citri is responsible for Huanglongbing, citrus stubborn disease. Phytoplasmas cause symptoms of decline and witches' broom, while a variety of citrus viroids, such as citrus Exocortis viroid or Hop stunt viroid, cause these diseases. These pathogens inhabit the phloem tissues, interfere with the movement of photo assimilates, and compromise root systems, eventually leading to a decline in yields, decline in fruit quality, and mortality of the trees. These phloem-restricted citrus diseases are known to be systemic, highly latent, and variably symptomatic and are ascribed to being effectively transmitted by insect vectors or non-sterile propagation media. Over the last few decades, changes in the incidence and the spread geographically of these diseases have risen steeply owing to increased global trade of plants, proliferation of vectors, and alterations in the ecology of vectors caused by climate change. The systems of weak certification, informal nurseries, scanty diagnostic infrastructure, as well as unsatisfactory management of vectors have also made citrus orchards in Asia, including Pakistan, susceptible to these pathogens. The present review synthesizes the existing knowledge of the world and regions on the citrus phloem-associated diseases with specific emphasis on the issue of pathogen diversity and disease development and its increasing menace to the citrus production in Pakistan and South Asia. There is a focus on disease drivers to facilitate proper surveillance and diagnosis, and combined management plans.

Keywords: Citrus; Phloem-associated diseases; Huanglongbing (HLB); Phytoplasma; Disease management

Citation: Fareed MA, Tahir A, Kello OJ, Mutti A, Imdad M, Iqbal S, Mushtaq MA, Shakoor K, Riaz T. 2026. Phloem Limited Citrus Pathogens in The 21st Century: Distribution, Diagnostics, and Management Strategies. Plant Science Horizons 1: e0006 https://doi.org/10.66835/psh.2026.e0006

Introduction

Citrus is among the fruit crops that are extensively grown worldwide, producing over 150 million tons annually with a significant contribution to world food and nutrition and international trade [1]. The citrus fruits play a prime role in the human body and the system of nutrition in the world through providing vitamin C, carotenoids, flavonoids and antioxidants [2]. The world citrus production is dominated by Asia which produces over 55 percent of the total output of citrus fruits with considerable volume of China, India, Pakistan, Iran and Thailand. Citrus farming is a horticultural economy in South Asia. [3]. The services by citrus exports generate a heavy flow of foreign exchange and earn living to millions of farmers and nursery workers as well as supply chain stakeholders [4]. Nevertheless, the citrus product in Pakistan is still significantly lower compared to developed citrus producing nations which is mostly caused by disease pressure, low quality of planting material and lack of appropriate orchard management practices [5].

Recent evaluations note that the perpetual production of citrus in Asia will be based on the success of disease prevention and early detection measures especially on the systemic vascular pathogens that pose a threat to the long-term of the orchard [6]. Phloem restricted pathogens which are also known as plasma-associated pathogens are the most devastating category of citrus pathogens as they are capable of colonizing phloem tissues systematically induce disruption of and hormone signaling as well as root development [7]. *Candidatus* Liberibacter spp. that cause that cause decline and witches broom symptoms and citrus viroid including Citrus exocortis viroid and Hop stunt viroid are all included in this group [6].

The most destructive of these diseases is Huanglongbing (HLB) which proceeds with the significant loss of production, malformations of the fruits, the system of roots discovers and the death of the tree and no cure exists [8]. Although phloem blockage,

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and starch accumulation seem to play a central role in disease development. Recent studies have also shown that phloem necrosis is central to the development of diseases [9]. Viroid infections provide also considerable economic consequences as they cause scaling to bark, stunting, low vigor and yield stats in the long run specifically with grafted citrus system [10]. In Pakistan and other countries citrus viroids and comorbidity with bacterial pathogens is probably underdiagnosed as routine indexing and other molecular diagnoses are underdeveloped [11]. Together phloem restricted pathogens pose a risk to the orchard life and are a significant challenge to the sustainability of citrus production both on the global and regional level.

The intensive development and redevelopment of citrus phloem related diseases in the last twenty years is a cumulative result of international trade in flora increases in vector population and climate changes [12]. International nursery plants transport of infected but asymptomatic plants has been found to represent one of the main routes of long-distance spread of phloem bound bacteria and citrus viroid [13]. Such a risk of disease establishment is significantly high in the developing states such as Pakistan because the uncertified planting material is widely used [5]. The spreading of diseases at the local and regional levels speeds up due to another transmitting phenomenon through vectors. The spread of the geographic range of the principal *Candidatus* Liberibacter asiaticus vector the Asian citrus psyllid has spread to new areas of Asia and the Middle East due to suitable climatic conditions and the increase in host availability [14]. Equally the abundance and efficacy of transmission of Spiroplasma and phytoplasma transmitted by leafhoppers have been found to be more robust and higher under hotter climatic conditions [15].

Climate change is a critical amplifier because it causes changes in the phenology of vectors, higher escape rates reduced incubation of pathogens and also by expanding disease friendly conditions [12]. According to recent modeling analyses it is estimated that South Asia will be more habitable by the psyllid populations and the citrus industry of Pakistan will be exposed to more frequent outbreaks of phloem diseases in the next several decades [6]. The review is a synthesis of existing research information on citrus phloem associated diseases around the world and more specifically discussing pathogen diversity, disease outbreaks and increasing danger to citrus production in Pakistan and South Asia. There is an emphasis on learning disease drivers to facilitate successful surveillance, diagnosis and integrated management strategies.

Classification of citrus phloem plasma diseases

Phloem limited bacteria

Phloem-limited bacteria are the most devastating type of pathogens of citrus globally because of their systemic colonization of sieve elements interference with the phloem transport and are effectively transmitted by vectors. The primary platforms of these bacteria are *Candidatus* Liberibacter species responsible to cause responsible to cause citrus stubborn disease and a number of lineages of phytoplasma responsible to cause citrus decline and witches broom disorders [16]. They are obligate host-dependent chemoheterotrophic bacteria associated with phloem tissues, making them difficult to cultivate, diagnose and manage

Candidatus Liberibacter species and Huanglongbing

The citrus greening disease is a disease related to three phloem limited bacteria *Candidatus* Liberibacter asiaticus, *Candidatus* Liberibacter africanus and *Candidatus* Liberibacter americanus. The most common and heat tolerant of them is *Candidatus* Liberibacter asiaticus and causes most epidemics in Asia, the Americas and some areas in Africa [17]. *Candidatus* Liberibacter africanus is mostly restricted to the cold areas in Africa whereas *Candidatus* Liberibacter americanus has been reported predominantly in Brazil and is regarded as less violent than *Candidatus* Liberibacter asiaticus [17]. The pathogen is spread mainly by the Asian citrus psyllid and the which contract this pathogen during the feeding process on diseased sap in phloem and spread it continuously to trees that are not infected [18]. *Candidatus* Liberibacter spp. multiply in vitro in the host in the phloem sieve elements and cause a series of events that include callose deposition, phloem plugging, accumulation of starch in leaves and progressive root decompensation [19]. The recent transcriptomic and anatomic studies showed that the root damage does not often manifest as a foliar symptom which is an indicator of the crypticity of HLB infection [20].

Spiroplasma citri and citrus stubborn disease

Another notable phloem limited bacterial disease is citrus stubborn disease which is caused by the wall-less mollicute *Spiroplasma citri* mostly in the warm and dry areas where citrus thrives [21]. In contrast to *Candidatus* Liberibacter, *S. citri* is culturable in the laboratory (but is limited to phloem tissues in plant) [22]. The bacterium is transmitted by the species of leafhoppers which are an important vector of the pathogen such as *Circuli fertenellus* feeding on the plant and transmitting the bacteria in a persistent form [23]. Some of the symptoms associated with infected citrus trees include retarded growth, retention of internes, erect leaves, shrubbery size, small, misshapen fruits with aborted seeds and color [24]. The temperature significantly affects the severity of the disease and high temperatures are conducive to the growth of pathogens and the manifestation of the symptoms [25]. According to the recent research *S. citri* infections may be latent over a long time and may complicate the diagnosis of the disease in the field and contribute to the persistence of the infection by infected planting materials [26]. Even though citrus stubborn disease is not as aggressive as HLB it may lead to great losses in yield and quality decline especially in unmanaged orchards and nurseries.

Phytoplasmas associated with citrus decline and witches broom

Phytoplasmas include Mollicutes as one of the classes of obligate wall-less bacteria that inhabit the phloem, besides being transmitted by sap-feeders which are predominantly leafhoppers and planthoppers [15]. Phytoplasma associated diseases have also been reported in citrus regions causing general decline, leaf yellowing, little leaf, witch broom and dieback and despite citrus not

being a primary host of phytoplasmas several diseases have been linked to the presence of phytoplasmas in citrus [27]. Witches broom disease of lime has had a terrible impact in the Middle East and South Asia and phytoplasma infections have resulted in the mass death of trees in Iran, Oman and adjacent areas [28]. There are a variety of phytoplasma entities related to citrus symptoms which have been recognized through a molecular mechanism of identification using the 16SrRNA gene sequencing technique which indicates the presence of a complex epidemiological situation with various vectors and reservoir host [29]. According to recent findings, climate warming and more active operations of vectors could contribute to phytoplasma transmission performance and overall, its geographic expansion to other citrus producing regions which include some regions of South Asia [30]. Systematic surveys of citrus related phytoplasmas are limited in Pakistan and the questions of under diagnosis and possible future outbreaks may arise [31].

Citrus viroid

Citrus viroid is the smallest known plants pathogen - circular non-coding RNA molecules of 250-400 nucleotides that can replicate autonomously and systemically in host plants and do not encode proteins. Despite viroid being simpler compared to the viruses they may result in critical yield suppression, growth malformations and economic damages in citrus particularly in case latent infections exist in commercial orchards and nurseries [32]. Citrus viroid are phloem-based agents that are distributed throughout the vascular system and then transmitted by the mechanic methods during grafting, use of contaminated instruments or stalking infected budwood [33]. Citrus exocortis viroid and Hop stunt viroids are the most researched and are both widespread worldwide and continue to become challenges in the management of citrus.

Citrus exocortis viroid

One of the earliest citrus viroids was Citrus Exocortis Viroid and it is still among the most significant citrus viroid cause of economic distress all over the world [34]. Citrus Exocortis Viroid can cause classical exocortis disease syndrome which is marked with scaling of the bark, lower trunk cracking, leaf chlorosis and stunting of trees especially when the hosts of this kind of disease are sensitive rootstock and Poncirus trifoliate and trifoliate hybrids [35]. On permissible rootstock interactions symptoms can be mild or nonexistent with the spread of the disease being unnoticed during nursery production [36]. The viroid replicates within the nucleus and chloroplast of host cells in a rolling and circular mechanism to produce linear multi Meric RNA which undergo processing into inflectional circular molecules [37]. The great stability of Citrus Exocortis Viroid RNA and its ability to be spread through mechanical methods imply that any small wound to citrus tissue can be used to spread it during pruning, budding or harvesting processes [38]. This renders extreme sanitation and indexing of nursery stock very essential in preventing viroid transmission [39].

The recent surveys based on high-throughput sequencing and RT-PCR detection have shown that the Citrus Exocortis Viroid is frequently present in mixed infections with other viroid or viral pathogens and can worsen symptom severity and make them difficult to diagnose [40]. Viroid surveillance is not a regular practice in South Asia and the prevalence and effects of Citrus Exocortis Viroid are therefore underestimated [41]. Certified clean planting material as well as systematically indexed is suggested to be used in integrated management to reduce long term orchard decline [42].

Hop stunt viroid

Another commonly spread citrus viroid is called hop stunt viroid and infects several and various hosts such as hop, grapevine, stone fruits and citrus [43]. Hop Stunt Viroid infection in citrus can be latent or lead to mild symptoms including stunting, loss of vigor malformed leaves and low set and size of round head fruits [44]. Citrus Exocortis Viroid and other viroid can also coinfect Hop Stunt Viroid to produce synergistic effects that increase the severity of the disease with resultant loss [45]. The Hop Stunt Viroid spread is either mechanical or graft-borne but the most significant avenue of epidemiology is represented by infected propagation materials [46]. Although viroid RNA has occasionally been detected in sap-feeding insects, aphid-mediated transmission of citrus viroids remains unconfirmed and is not considered a major epidemiological route compared with grafting and mechanical transmission. Even though the transmission through insects in citrus due to Hop Stunt Viroid has not been conclusively proven [36].

Recent diagnostics methods including RT-PCR real-time quantitative RT-PCR and high-throughput sequencing have enhanced the sensitivity of detection and have made it possible to specifically identify on the viroid species in asymptomatic trees [47]. These molecular technology tools have become critical in the efficient viroid surveillance and especially in the nursery stock certification programs. Even though significant studies have been conducted on the viruses and other bacterial diseases in citrus globally full surveys of Hop Stunt Viroid in predominant citrus areas such as Pakistan, India and other South Asian countries are still not established which signifies the existence of a big knowledge gap in the region regarding disease and its control measures [48]. Diagnostic capacity enhancement and the adoption of clean-plant program will also be part of the measures that will be taken to mitigate the long-term effects of Hop Stunt Viroid in citrus (Figure 1).

Disease symptoms and pathogenesis

Huanglongbing

Huanglongbing or citrus greening disease is the most devastating phloem related disease of citrus globally. It is mostly linked to the *Candidatus* Liberibacter asiaticus a phloem restrains and culturable bacterium that is transmitted primarily by the Asian citrus psyllid [18]. Commercial citrus species and cultivars are all infected by the disease that results in gradual degeneration and subsequent demise of the trees. Yellow shoots and the mottle of the leaf are some of the first and most typical signs of HLB that are indicative of significant disturbances in the work of the phloem and host physiology [49].

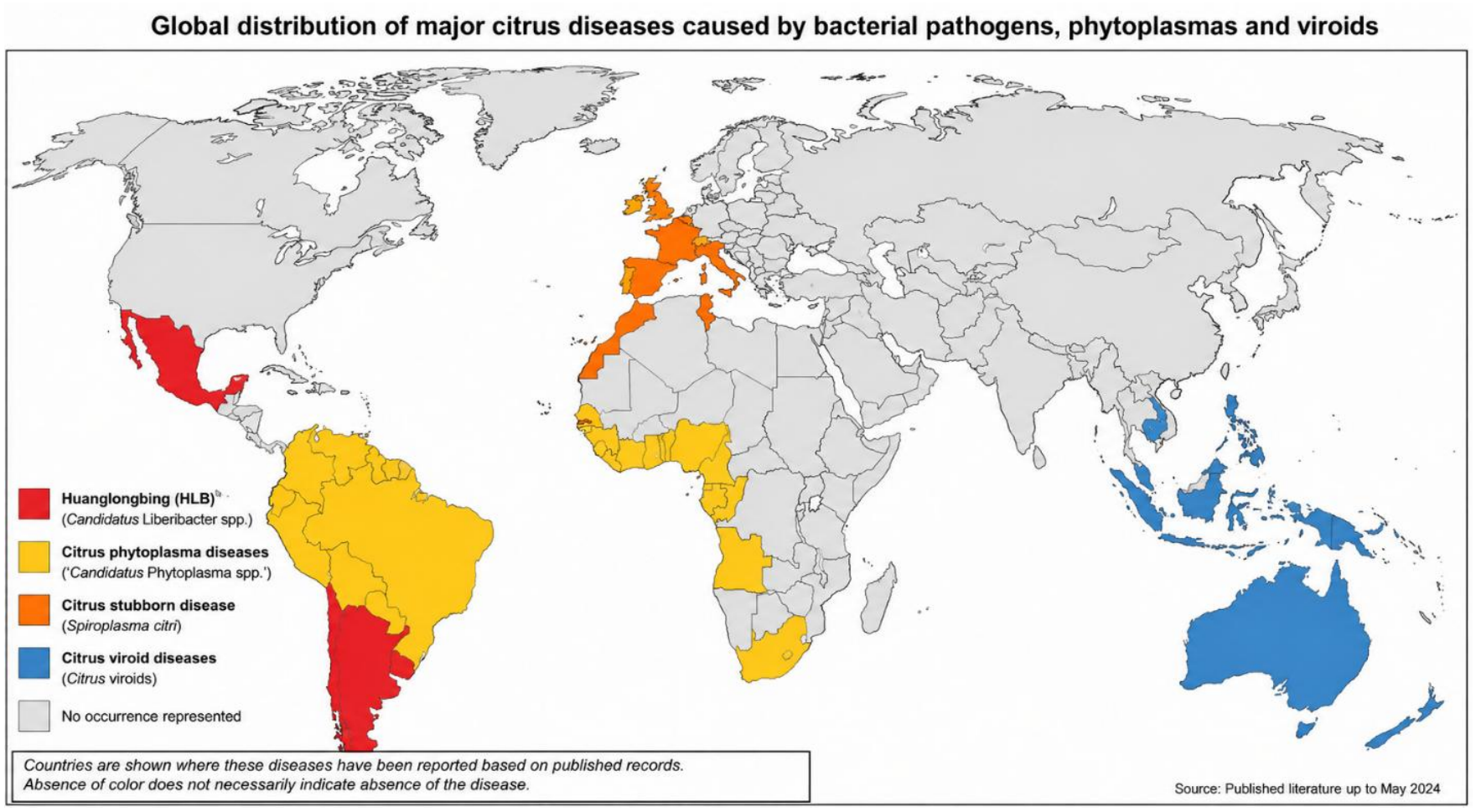


Figure 1. Global distribution of major citrus diseases caused by bacterial pathogens, phytoplasmas, and viroids. Countries where the diseases have been reported are highlighted according to disease type: Huanglongbing (HLB, citrus greening disease) (red), citrus phytoplasma-associated diseases (yellow), citrus stubborn disease caused by *Spiroplasma citri* (orange), and citrus viroid diseases (blue). Countries shown in gray indicate regions for which no occurrence.

Table 1. Major Citrus Phloem Limited Pathogens and Characteristic

Pathogen	Type	Disease	Symptoms	Vector
Candidatus Liberibacter asiaticum	Bacterium	HLB	Blotchy mottle	Citrus psyllid
Spiroplasmacitri	Bacterium	Citrus stubborn	Stnting &Twisted fruits	Leafhopper
Phytoplasmas	Bacterium	Citrus decline	Yellowing, dieback	Leafhopper
Citrus Exocortis Viroid	Viroid	Exocortis	Bark scaling	Graft & Mechanical
Hop Stunt Viroid	Viroid	Stunting	Reduced growth	Graft & Mechanical

Yellow shoots

One of the first symptoms of HBL to be manifested is the yellow shoots otherwise known as yellow branch or yellow shoot syndrome which tend to manifest as focal chlorosis on one or multiple of the shoots in the canopy [50]. The leaves on these shoots are pale yellow have decreased leaf size and have compromised growth in comparison to the adjacent normal looking branches. The imbalanced distribution of the symptoms of the canopy describes the discontinuous colonization of *Candidatus* Liberibacter asiaticus in phloem tissues [51]. Pathogenetic observations depicted that the formation of the yellow shoot is closely linked with the phloem blockage in response to the deposition of the callose and collapse of the sieve elements causing the inability to conduct the photoassimilates and nutrients [52]. The localized starvation of carbohydrates causes the degradation of chlorophyll and decreased photosynthesis in the form of shoot yellowing [53]. Also, recent transcriptomic experiments indicate that *Candidatus* Liberibacter asiaticus infection alters the expression of host genes that involve nutrient transport, hormone communication and defense responses and aggravates chlorosis in shoots [54].

Blotchy mottle

The most typical symptom of HLB that is diagnostic of the disease is blotchy mottle which consists of asymmetrical and irregular spots of yellow and green on older leaves [55]. A bilateral symmetry across the midrib is also a critical feature of field differentiation of HLB unlike nutrient deficiencies which are known to cause bilateral symmetry [56]. The growth of blotchy mottle is directly connected to an extreme phloem dysfunction caused by Candidatus Liberibacter asiaticus infection. It has been established using microscopic and physiological analysis that starch builds up in diseased leaves because of a defect in phloem export resulting in the distortion of chloroplasts, disorganization of thylakoid membranes and a drop-in photosynthetic activity [57]. This glottalization of starch disrupts the synthesis and accumulation of chlorophyll that causes the emergence of mottles [58]. The recent statements give a hint that the appearance of blotchy mottle is often observed in the case the damage to the roots has already been sharp which shows that foliar symptoms are a later manifestation of systemic infection [59]. This late outlook of the symptoms makes early diagnosis of the disease more difficult and increases silent distribution of HLB in the citrus orchard especially in a country with limited diagnostic power like South Asia and Pakistan [5].

Citrus stubborn disease

Citrus stubborn disease is a chronic phloem-associated infection caused by the wall-less bacterium *S. citri* that belongs to the family of Mollicutes. The infection is vastly prevalent in warm and dry and semi-arid areas covered with citrus plants and is caused by leafhopper vectors especially *Circulifer tenellus* [60]. Citrus stubborn disease can cause long-term growth deformity, lower fruit quality and massive losses of yield compared to HLB but unlike HLB disease can scarcely result in rapid death of the trees; it tends to affect young trees or nurseries, which leads to persistent occurrence of the disease [24].

Twisted small fruits

Small, misshapen and twisted fruits are considered to be one of the most frequent and cost-efficient symptoms of citrus stubborn disease. Infected fruits can be undersized, long or smooth sided with an abnormal rind texture and bad coloration and do not turn their regular size even at their maturity [61]. These distortions significantly lower the market value and they are one of the significant contributors of economic loss in infected orchards. Pathogenetic studies suggest that twisting and size decrease of fruits is caused by impairment of the transport of phloem and change in hormone balance in the growing fruit. Intrusion of *S. citri* causes invasion of phloem tissues that nourish immature fruits resulting in a defective translocation of carbohydrates and hindrances in cell division and growth [62]. In the recent physiological studies, it has been revealed that infected fruits have lower sugar buildup, impaired vascular differentiation and disruptive auxin and gibberellin signal transduction which are combined to produce deformed fruit morphology [63]. Symptom expression is also worsened by other environmental factors such as high temperature. The warm temperature encourages the growth of the pathogen and worsens the defects of the fruits which is why the stubborn disease is widely spread in the Asian countries and the Middle East and the part of Africa with hot climates [64].

Abnormal seed formation

The other hallmark symptom of the citrus stubborn disease is abnormal seed development. Products of infected trees often have aborted shriveled and not necessarily developed seeds in most cases they do not have them at all [65]. These malformations are strictly connected with disturbed reproductive development induced by infection of phloem tissues supplying floral organism and developing embryo by *S. citri*. At the pathogenetic level disturbed embryo formation is linked with the distortion of nutrient flow and hormonal signals the normal ovule fertilization and developing embryo needs. It has been shown that *S. citri* infection also causes decreased translocation of assimilates to reproductive tissues, aborting embryos and with low seed viability [66]. Moreover *S. citri* is involved in interference with gene expression linked to floral growth and seed maturation by molecular evidence as well leading to reproductive failure further [67]. Practically, the seed abnormal formation is an excellent diagnostic feature of stubborn disease when used in association with deformation of the fruit and poor color. Nonetheless field diagnosis may be complicated by the symptom overlap with nutrient deficiencies or other stresses which highlights the necessity of the molecular confirmation of the diagnosis using PCR based assays [68].

Phytoplasma associated decline

Another limiting factor to citrus production that has not yet reached the states of awareness is phytoplasma associated citrus decline which is a local menace to citrus production across various parts of Asia, the Middle East and certain regions of Africa. Phytoplasma are phloem restricted wall-less and obligate bacteria of the Mollicutes that are propagated by biting insects mostly leafhoppers and planthoppers [15]. Citrus is no longer viewed as a key host of phytoplasma yet infection may still cause severe physiological dysregulations, growth malfunctions and eventual deterioration especially in favorable environment conditions [61].

Leaf yellows

This is partially due to the misfortune that most symptoms of phytoplasma infection in citrus appear first and are similar to leaf yellows. Affected trees have generalized chlorosis, leaves of yellow to pale green color, small leaf size, early leaf senescence and many of them tend to appear similar to nutrient deficiencies [15]. Yellows on leaves caused by phytoplasma are generally more uniform across the leaf surface as well as the rest of the canopy as opposed to HLB related blotchy mottle although dashes can also be affected by host genotype phytoplasma strain and environmental conditions [69]. The pathogenesis of the leaf yellows is in the colonization of the sieve elements by Phyto plasma and disrupts the transport of the phloem as well as changes the source-sink relations in the plant. Out of the recent physiological findings is the fact that Phyto plasma infection disrupts carbohydrate allocation, decreases the production of chlorophyll and changes the metabolism of nitrogen thus resulting in chronic chlorosis and compromised photosynthetic capacity [70]. Moreover, phytoplasma release proteins with effector functions that inhibit host defense systems and alter hormones signaling pathways especially auxins and cytokinins which also lead to yellowing of the leaves [71].

Witches broom

The appearance of a witch's broom is a disease hallmark and diagnostic characteristic of phytoplasma infection which is an excessive growth of shoots, shortening of internodes and broom-like collections of thin and upright branches [30]. Witches broom causes extreme canopy defect in citrus and the related species at the expense of flowering and subsequent decline of the infected tree. Recent reports have revealed that phytoplasma effectors including *SAP11* and proteins of a similar genes interact with transcription factors causing the abnormal proliferation of shoots and loss of apical control [70]. These growth and development interferences redirect the integrates in reproductive development and hence limit the fruit production and hasten the erosion. The broom disease of lime is one of the most dreadful phytoplasma related diseases that have caused immense destruction in the Middle East and even South Asia where orchards have been rapidly abandoned and trees killed without delay [72]. Climate factors that provide favorable conditions

to the activity of vectors as well as the occurrence of alternative host plants have predetermined persistence and reproduction of phytoplasma in citrus growing areas [73].

Viroid induced disorders

Citrus viroids are the smallest size of plant pathogen they are circular non coding RNA molecules that replicate autonomously in host cells. Although not very complex viroid may systemically infect citrus phloem tissues and lead to chronic growth abnormality, bark abnormalities, leaf defects and yield losses [74]. Bark cracking which is a complication of Citrus Exocortis Viroid and leaf bending which is commonly a symptom of Citrus Bark and Leaf Viroid are some of the most striking disorders caused by viroid. The latent infections in the cases of these disorders are not only causing low productivity but make nursery management difficult.

Bark cracking

The most common effect of Citrus Exocortis Viroid infection is called exocortis which is a characteristic symptom of this disease particularly of trifoliate hybrids [75]. Common symptoms of infected trees are vertical cracks or scaling of the lower trunk and main branches and sluggish shoot development, decreased vigor and slight chlorosis of leaves. The disease progresses due to the viroid replicating the phloem and the surrounding cambial cells and this results in deviations in the way cell division and lignification occur in the bark. This disturbance induces mechanical strain which makes the bark tear and flake. It has been demonstrated through experimental research that Citrus Exocortis Viroid changes the expression of host genes associated with metabolism in the cell wall, hormone signaling and stress responses all of which are associated with these deformities in the bark [36]. Cracking of barks is very acute in young orchards and plantations in nurseries and timely identification of this disease is important in managing the disease.

Leaf bending

The most notable symptom of the Citrus Bark and Leaf Viroid infection is the leaf bending, twisting or down curling [76]. Plants with the disease tend to have leaves that are twisted or asymmetrically bent, small, chlorotic and slowly stunted canopy in the long run. The disease occurs due to the fact that Citrus Bark and Leaf Viroid distorts the transport of phloem and affects hormonal reactions specifically auxin and gibberellin involved in normal leaf growth and orientation [77]. Replication of viroid in phloem tissues causes release of stress and abnormal growth of cells leading to the typical bending of the leaf. Symptoms are aggravated in most instances by coexistence with other citrus viroid including Citrus Exocortis Viroid [78]. Leaf curvature mainly of delicate rootstock-scion unions, can be used as a convenient field indicator of Citrus Bark and Leaf Viroid infection, but molecular verification by RT-qPCR throughput sequencing is suggested due to the manifestation of similarities in the symptoms with nutrient deficiencies or other viruses [33]. In **Figure 2** present signs that show up with HLB in citrus plants. The most recognizable symptoms of HLB are irregular blotchy mottle patterns and asymmetrical yellowing of citrus leaves, distinguishing HLB from nutrient deficiencies that generally produce more uniform discoloration. Fruits that are infected with HLB show distortion, weird morphology, and growth that really slows down. Later on, a strong chlorosis along with mottled leaf patterns, like the whole leaf looks off appears. HLB is driven by *Candidatus* Liberibacter species, and it is seen as one of the most damaging diseases putting citrus growing at risk on a worldwide level.

Pathogen biology and transmission

The spread of phloem limited pathogens in citrus, including bacteria, phytoplasmas and viroid is strongly influenced by vector biology. Transmission by sap feeding insects enables pathogens to bypass host surface defenses and establish systemic infections. Understanding vector pathogen interactions feeding behavior and population dynamics is critical for disease management and epidemiological modeling [61].

Transmission and Disease cycle

Insect vectors

Diaphorina citri

The Asian citrus psyllid is the primary vector of *Candidatus* Liberibacter asiaticus the causal agent of HLB. This insect is highly efficient in acquiring and transmitting *Candidatus* Liberibacter asiaticus due to its phloem feeding habit and widespread presence across citrus growing regions in Asia and the Americas and the Middle East [81]. The acquisition of *Candidatus* Liberibacter asiaticus happens during feeding on the trees that are infected followed by multiplication of the bacterium in the psyllid that migrates to the hemolymp and later to the salivary glands. It takes a latent period before the psyllid can infect healthy plants with the pathogen [79]. *Candidatus* Liberibacter asiaticus is circulative and persistent transmission using nymphal stages is more effective in obtaining the bacterium than adults resulting in populations of young psyllids being especially crucial in early disease dissemination [82]. Moreover, *Candidatus* Liberibacter asiaticus is able to control psyllid dispersal and frequency that increasing the epidemic potential of HLB [83]. The biology of *D. citri* is critical to adopting effecting management measures such as chemical management and biological management involving the use of parasitoids that feed on cyanidium spores and eliminate infected trees to prevent sources of inoculums. Insect vectors sort of have a big epidemiological part when it comes to spreading citrus phloem associated pathogens. Psyllids and leafhoppers, they pick up pathogens while feeding on

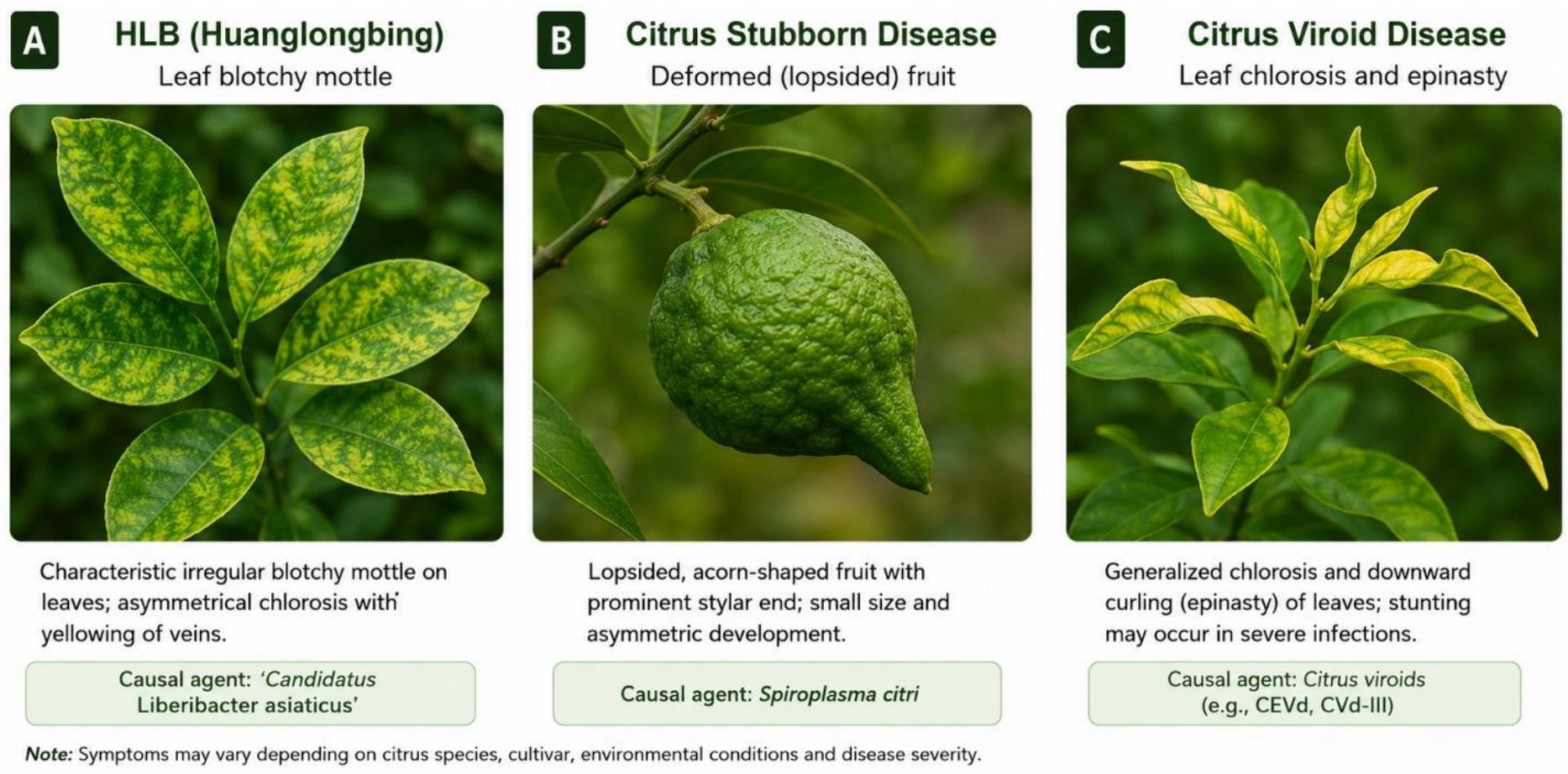


Figure 2: Representative symptoms of Huanglongbing (HLB) in citrus. (A) Citrus leaves exhibiting characteristic blotchy mottle and asymmetric chlorosis, a diagnostic symptom of HLB infection. (B) HLB-affected fruit showing severe deformation, irregular shape, and impaired development. (C) Advanced leaf chlorosis and mottling associated with HLB infection. HLB is caused by *Candidatus* Liberibacter spp. and is considered one of the most destructive diseases affecting citrus production worldwide.

infected phloem tissues and then, later they push those pathogens into healthy plants in a persistent circulative way. The nymph stages tend to be more efficient for acquisition than adults. This is mainly because they just feed longer, and keep at it. Also, how vectors move between orchards, their liking for young flushes and their overall strong reproductive output, all that really speeds up disease progression. On top of that, it can happen that certain pathogens tweak the vectors feeding habits and even their dispersal activity, so transmission turns more efficient and epidemics can develop faster.

Leafhoppers

By far Spiroplasma citri is spread by leafhoppers as well as several phytoplasma specializing on citrus [25]. Their feeding strategy and phloem specialization profit an effective way of pathogen resourcing and inoculation.

Acquisition and transmission

S. citri is acquired during phloem feeding and persists in leafhopper vectors for life. Transmission is persistent-circulative meaning the pathogen circulates within the insect and is transmitted during subsequent feedings [22]. Phytoplasmas are similarly acquired by leafhoppers and transmitted in a persistent manner. Vector competence depends on both the leafhopper species and the phytoplasma strain [15]. Phloem limited pathogens may manipulate leafhopper feeding behavior increasing the frequency and duration of probing thereby enhancing transmission efficiency [84]. Leafhopper mediated transmission facilitates local and regional spread of stubborn disease and phytoplasma associated citrus decline. Environmental factors particularly temperature and vector abundance strongly influence disease incidence [24].

Mechanical transmission

Mechanical transmission plays a critical role in the spread of phloem limited pathogens in citrus particularly viroids and certain bacterial pathogens. Unlike insect mediated transmission mechanical routes involve the direct transfer of pathogens through wounds created during propagation, pruning and other cultural practices [56]. Grafting infected bud wood onto healthy rootstock is the most common pathway for disseminating *Candidatus* Liberibacter spp., *S. citri* and citrus viroid. Systemic infection can establish within weeks, depending on pathogen load scion rootstock compatibility and environmental conditions [85]. Tools such as pruning shears, knives and other instruments can also carry pathogens from infected to healthy plants, with studies demonstrating that even low concentrations of viroid RNA or bacterial cells are sufficient to establish infection if introduced into phloem tissue [86]. The mechanical transmission is very critical when nursery propagation of various scion varieties and rootstocks and certification of viroid and pathogen free programs are not provided which can increase the high occurrence of illnesses in emerging orchards considerably [75]. To avoid the mechanical transmission, proper sanitation of instruments and close attention to grafting material should be followed. Moreover, nursery stock has been successfully treated with heat therapy and in vitro tissue culture proliferation to get rid of viroid infections [87].

Environmental influence

Climate change gets treated like a major factor shaping the epidemiology of citrus phloem-linked diseases. As temperatures go up, precipitation gets weird in new ways and droughts hang on longer, it changes vector life, how pathogens multiply, and how susceptible the host trees become. In general, warmer surroundings help vectors in a bunch of ways, like better reproduction, stronger feeding activity, and more movement, so insects such as *D. citri* and various leafhoppers end up spreading disease more efficiently. That then pushes transmission of HLB citrus stubborn disease and phytoplasma-linked decline. Higher temperatures can also speed up how *Candidatus* Liberibacter *asiaticus* and *S. citri* multiply inside the plant tissues and also within the insect vectors. On top of that, when the climate changes the places where vectors can live their habitat range may stretch and then diseases such as HLB and citrus stubborn can spread into citrus regions that were basically untouched before. And there's more, the environmental strain from climate change might make the host plants defenses less strong which then raises disease susceptibility and even makes symptoms look worse than they would otherwise [88].

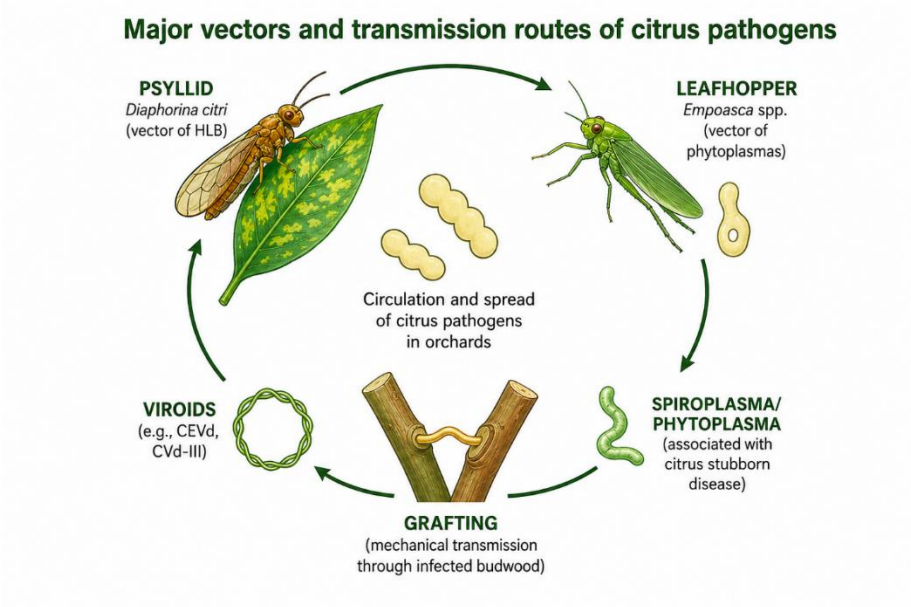
Temperature stress

The temperature is a crucial factor in biology and epidemiology of citrus pathogens which are phloem limited. It puts into effect pathogen multiplication and other bacteria like *Candidatus* Liberibacter *spp., S. citri* and phytoplasmas multiply according to ambient condition. As an example, *S. citri* grows faster at moderate to high temperature resulting in a higher level of symptom without young citrus trees [88]. Temperature also controls the abundance of vectors, feeding patterns and dispersal. HLB transmits more rapidly as the Asian citrus psyllid has a higher population increase and movement rate in the. On the other hand, high temperatures above can shorten the duration of survival of control of any pathogen in both the host and the vector [16]. Besides that, temperature stress also can be a problem to enhance symptom expression. The signs of Exocortis activated by Citrus Exocortis Viroid are more sensitive in warm and dry seasons because viroid replication is increased and the host responds to a greater level of stress [89].

Host susceptibility

Genetic and physiological factors have a great effect on host susceptibility to phloem limited pathogens. The rootstock and scion genotype are also of significant importance where *Poncirus trifoliate* has the highest sensitivity to Citrus Exocortis Viroid whereas some sweet orange cultivars are only partially tolerant. In a similar manner, there are citrus species that show reduced development of HLB symptoms in situations where they get systemically infected by *Candidatus* Liberibacter *asiaticus* [85]. The vulnerability is also related to age and physiological condition of the plant since young actively growing trees are more likely to be infected and develop severe symptoms than mature trees [90]. The host susceptibility can also be aggravated by environmental and physiological stress such as lack of nutrients or previous exposure to a pathogen. These may point to the significance of using nontolerant rootstocks, cultivars, and practices that reduce stress to reduce the effects of pathogens and extend the lifespan of orchards **Figure 3** [91]. This overall contributes to the setting up of disease and its long-term survival in citrus production regions [90].

Figure 3. Major vectors and transmission routes of citrus pathogens. Schematic representation of the principal pathways involved in the



dissemination of economically important citrus pathogens. Huanglongbing (HLB)-associated bacteria (*Candidatus* Liberibacter spp.) are primarily transmitted by psyllid vectors, particularly *Diaphorina citri*. Citrus phytoplasmas are disseminated by phloem-feeding leafhoppers, whereas citrus stubborn disease is associated with the bacterium *Spiroplasma citri*. Citrus viroids, including Citrus exocortis viroid and Citrus viroid III, are predominantly spread through infected propagation materials, grafting practices, and contaminated horticultural tools. The circular arrows illustrate the continuous movement and spread of pathogens within citrus orchards through vector-mediated transmission and vegetative propagation, contributing to disease establishment and long-term persistence in citrus-growing regions.

Diagnostic techniques

Diagnosis of citrus stubborn disease, viroid induced disorders and HLB is essential to control the citrus phloem limited pathogens. Among the methods used to make diagnostic decisions it is possible to distinguish between the molecular and serological techniques.

Molecular diagnostics

The most sensitive and specific measures of identifying phloem restricted pathogens in citrus are molecular methods. The detection of *Candidatus* Liberibacter spp., the causal agent of HLB, is implemented with traditional PCR methods as well as with quantitative real-time PCR which allows not only to identify the presence of the bacterium but also to quantitatively monitor the load of the bacteria which is valuable data regarding the severity of the infection and the risk of transmission and the design of the primers varies depending on the 16S rRNA or individual prophage gene [92]. Next generation sequencing technologies enable simultaneous detection of multiple pathogens including Liberibacter spp., Spiroplasma, phytoplasmas and viroid making it especially useful in cases of mixed infections where traditional methods may fail to identify infections [80]. These molecular techniques offer high sensitivity and the ability to identify latent infections.

Serological diagnostics

Serological assays detect pathogen presence through antigen antibody interactions. Enzyme linked immunosorbent assay (ELISA) is commonly used for the detection of *S. citri*, the causal agent of citrus stubborn disease with antibodies raised against *S. citri* antigens enabling rapid screening of orchard and nursery samples [62]. While ELISA is effective for bacterial pathogens such as *S. citri*, it is generally not suitable for viroid because of viroid lack protein components. Serological methods are relatively very low cost, simple and suitable for high throughput screening but they are less sensitive than molecular techniques and may produce false negatives in early infections or in plants with low pathogen titers.

Field diagnosis

Field based diagnostic approaches are essential for early detection particularly in regions with limited access to laboratory facilities. Visual observation of characteristic symptoms such as yellow shoots and blotchy mottle in HLB, twisted fruits in stubborn disease, witches' broom associated with phytoplasmas, bark cracking caused by Citrus Exocortis Viroid and leaf bending can provide preliminary identification of infections [61]. Surveillance of insect vectors including *Diaphorina citri* and leafhoppers offers indirect evidence of disease risk with sticky traps and population counts informing management decisions and helping prioritize samples for laboratory testing [93]. These field-based methods are immediate, cost effective and enable rapid response.

Table 2: Comparison of Diagnostic Tools

Diagnostic Tool	Target	Advantages	Limitations	References
PCR	Bacteria	High sensitivity	Requires lab	[119]
RT-PCR	Viroids	Accurate detection	Expensive	[117]
qPCR	Bacteria	Quantitative	Skilled personnel needed	[118]
LAMP	Bacteria	Field-friendly	Lower specificity	[112]
ELISA	Bacteria	Cheap	Less sensitive	[19]
NGS	All pathogens	Detects unknown pathogens	High cost	[116]

Disease epidemiology and spread

The epidemiology of citrus pathogens that feed on the phloem's is very important in developing strategies to control the pathogen. Human activity and the ecology of the vector determine the proliferation of HLB, citrus stubborn disease, phytoplasma associated decline and the violation of the ecological sphere of viroid. Nurseries, plant material movement and release of vectors are considered to be some of the key contributors in local and long-distance transmission of these pathogens according to epidemiological studies [94].

Role of infected nurseries

One of the main origins of the inoculum of pathogen in citrus orchards is nurseries because the materials of scions and rootstock that are infected help in spreading the phloem limited pathogens over a long distance. The primary route of bringing, *S. citri*, phytoplasmas and viroids into new orchards is via grafting of infected budwood [95]. Most phloem-based infections can be without symptoms in young nursery plants. This permits the free flow of infected material and makes it more likely that [96]. The certification of nursery stock as free of any pathogens, habitual testing with PCR and rigorous sanitation methods are needed to ensure the overall health of the orchards and reduce the transmission of these pathogens which are devastating [97].

International trade

The intercontinental transfer of pathogens of phloem limited pathogens has been enabled by globalization and international transmission of citrus planting material and ornamental plants. International trade of infected budwood and seeds has been suggested in the importation of HLB in the Americas in addition to the dissemination of Citrus Exocortis Viroid in Asia and Africa [98]. The nature of the latent infection and inappropriate sampling results in the fact that viroid and bacterial pathogens are often not detected by quarantine and thus on the contrary, complicate the process of preventing the spread of organisms [99]. The above issues demonstrate that balanced phytosanitary laws and stringent coming testing systems will play a vital role in reducing the chances of new epidemics and guarantee safe worldwide commercialization of citrus germplasm [100].

Vector ecology

The local and regional distribution of phloem limited citrus pathogens highly rely on the nature of vectors which are greatly dependent on temperature and availability of the host and seasonal dynamics. The vectors of HLB and the vectors of stubborn disease and phytoplasma multiply intensely at temperatures ranging between 25 and 30 °C which allows the virus to spread rapidly [101]. Extreme elevated temperatures of over 35°C or lower temperatures of less than 15°C may on the other hand diminish the survival and feeding of vectors which will slow down the development of diseases temporarily. The young shoots and leaves are favorable to psyllids and leafhoppers to enhance the acquisition or inoculation efficiency of the pathogen whereas their migration patterns and dispersal ranges as well as reproductive stores have a direct impact on epidemiology [102]. Moreover, some of the pathogens are capable of altering the actions of the vectors such as *Candidatus* Liberibacter asiaticus stimulates the feeding and dispersal of psyllids which increases the transmission of HLB [103].

Integrated disease management

Management of the phloem limited citrus pathogens such as HLB, citrus stubborn disease, phytoplasmas and viroid needs an integrated strategy that is holistic. IDM integrates cultural, vector management and resistant rootstock that technological

interventions in the geographical reduction of the pathogen incidence and the limitation of the vectors [60].

Cultural control

The cultural practices are the sources of integrated disease management by reducing the inoculums sources and the lack of pathogen spread. Inoculum pressure is reduced because infected trees are removed soon before becoming symptomatic [104]. Certified rootstock and pathogen-free budwood are required when introducing a new orchard to help eliminate any HLB, viroid and other pathogens that are susceptible to phloem [105]. Nursery environments include it as an important way of reducing infection by mechanical transmission of viroid and bacterial pathogens through cleaning of grafting tools, pruning equipment and propagation areas. So that healthy plant material can be used in the establishment of orchards [106].

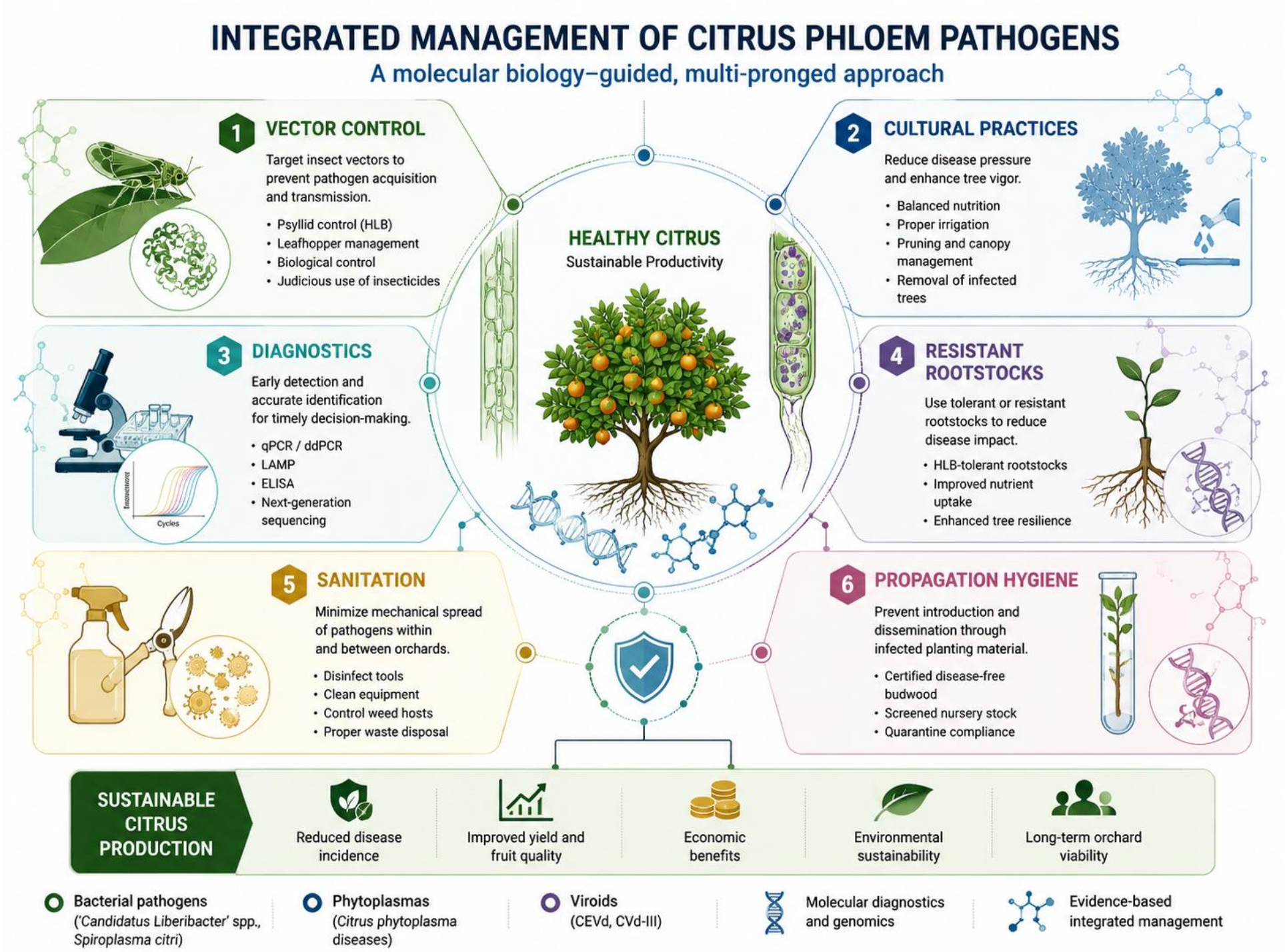


Figure 4. Integrated management strategies for citrus phloem-associated diseases. Integrated disease management framework illustrating the major approaches used to prevent, monitor, and control citrus phloem-associated pathogens, including *'Candidatus Liberibacter'* spp. (Huanglongbing, HLB), *Spiroplasma citri* (Citrus Stubborn Disease), phytoplasmas, and citrus viroids. Effective management relies on a combination of vector control to suppress insect vectors such as psyllids and leafhoppers; cultural practices including orchard monitoring, nutritional management, and removal of infected trees; diagnostics for early detection and pathogen surveillance; use of resistant or tolerant rootstocks to improve disease resilience; implementation of strict sanitation measures to reduce pathogen spread through contaminated tools and equipment; and propagation hygiene through the use of certified disease-free budwood and nursery stock. The integration of these complementary strategies forms the foundation of sustainable disease management programs aimed at reducing pathogen dissemination, minimizing economic losses, and maintaining long-term citrus productivity.

Vector management

The application of a sanitary cleaning of grafting tools and pruning equipment to minimize mechanical transmission of viroid and bacterial pathogens is the primary way to reduce transmission of the diseases [107]. Insect vectors Suppression of insect vectors is also important in control of insect carried pathogens. The use of systemic and contact insecticides focused on psyllids and leafhoppers will go a long way in suppressing instances of HLB, citrus stubborn disease and phytoplasma related decline [108]. The use of biological agents like the Parasitoid has also shown to be effective against *D. citri* and this means a sustainable way of

suppressing its population in case integrated into management initiatives [109].

Resistant rootstocks

Huanglongbing and viroid tolerance Citrus rootstocks developed as long-term resistant to commercial orchards in particular development of new citrus rootstock that is partially resistant to HLB and viroid. These hybrids are an integration of disease resistance and environmental adaptiveness which have displayed minimized manifestation symptoms and higher productivity [110]. Resistance breeding aims at preserving phloem integrity and repelling the feeding of vectors. These tolerant rootstocks are finding use more and more in orchards in Asia and America.

Advanced methods

New biotechnological solutions provide could be used as an extension of sustainable management of citrus diseases. Netting can also be used as a protective insect proof surface that restricts the access of vectors and thus acts like warranty protecting not only *D. citri* and leafhoppers but also improving the usefulness of integrated management programs Figure 4 [111]. In practice, successful control seems to rely on a mix of multiple methods vector management to keep insect vectors at lower levels including psyllids and leafhoppers then cultural measures too such as routine orchard inspections more balanced nutrient management and the removal or eradication of infected plants. Also, sensitive diagnostic tools matter or quick detection and surveillance and then resistant or tolerant rootstocks are used to improve plant endurance. Finally, strict sanitation rules help a lot because they reduce pathogen spread via contaminated tools equipment and even propagation materials. All these things when combined support long-term disease suppression and better overall citrus health.

Research gaps and future perspective

Longitudinal studies are the top priorities of future research to address the gaps, mapping pathogen diversity, learning the risks of different strains and exposing novel hazards. Research on the molecular interactions between vectors and pathogens or their hosts such as effectors and the immunity of vectors will be beneficial in increasing the targeted practices of managing the vectors. Portable, point of care diagnostics including but not limited to LAMP assays, CRISPR based biosensors as well as nano-pore sequencing would allow real time detection of pathogens in orchards and nurseries. The acquisition of resistant or tolerant citrus varieties can be fastened by breeding programs that embrace CRISPR mediated genome editing, marker-assisted selection and the traditional hybridization. The use of proactive disease management information based on model predictions that include climate information, movement of vectors and nature of pathogen biology will be based on the information. In Pakistan and Thailand, it is essential to enhance disease surveillance, availability of certified material of plant material free of pathogens and establishing grower awareness initiatives to reduce the effects of phloem limited pathogens and provide an environmentally friendly production of citrus.

Conclusion

Phloem connected citrus pathogens, like *Candidatus* Liberibacter spp., *S. citri*, phytoplasmas and citrus viroids really pose a serious danger to world citrus output. They colonize the phloem tissues systemically, and because spread happens well through vectors plus the whole infection can stay latent for a while managing the disease becomes very hard. In other words, it's kind of a tricky situation that resists easy control. Recent developments in molecular diagnostics, epidemiological research and combined approaches towards disease management have enhanced our perception of spread and control of the pathogens. A gap in knowledge persists especially on pathogen diversity interactions between vectors and pathogens and climate change effects on the dynamics of the diseases. The adoption of clean nurseries which managing the vectors and detection of the disease at early and accurate stages. The development of resistant or tolerant citrus varieties should be prioritized in the future. The combination of traditional methods of management with modern biotechnologies including CRISPR mediated gene editing diagnostics and insect proof methods can contribute to a decrease in disease effects to a significant extent. Surveillance of diseases, certification program and awareness of the growers in areas like Pakistan and Asia will be significant to ensure the production of citrus in an environmentally friendly manner. Finally, the implementation of scientific innovation and active policymaking is needed in a multidisciplinary manner to curb the threat of phloem limited citrus pathogens and to ensure the sustainability and continued productivity of global citrus orchards.

Data availability

Not applicable

Acknowledgments

Not applicable

Conflict of interest

The authors declare that they have no conflict of interest.

Author Contribution

The authors confirm contribution to the paper as follows: M.A.F, A.T, A.M, and O.J.O: writing-original draft, conceptualization, writing, review, and editing. M.I, S.I and M.A.M: review and editing, conceptualization and reviewing. K.S and T.R: Supervision, reviewing, editing and manuscript polishing. All authors discussed the content, reviewed the results and approved the final version of the manuscript.

Funding

Not applicable

Ethics Statement

Not applicable

Publisher's note

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Generative AI Statement

The authors declare that generative artificial intelligence (AI) was not used in the scientific content creation, analysis, or interpretation of this manuscript. Any AI-assisted language editing or formatting support, where applicable, was carefully reviewed and verified by the authors.

Dates

Received 30 April 2026; Revised 08 June 2026; Accepted 11 June 2026; Published online 20 June 2026

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