CAPS Datasheets provide pest-specific information to support planning and completing early detection surveys.

‘*Candidatus* Phytoplasma ziziphi’

Scientific Name

'*Candidatus* Phytoplasma ziziphi' Jung et al. 2003

Synonyms:
None

Common Name

Jujube Witches' Broom Phytoplasma

Type of Pest

Phytoplasma

Taxonomic Position

Class: Mollicutes, **Order:** Acholeplasmatales, **Family:** incertae sedis - Family II
Genus: ‘*Candidatus* Phytoplasma’

Pathogen Recognition

This section describes characteristics of the organism and symptoms that will help surveyors recognize possible infestations/infections in the field, select survey sites, and collect symptomatic material. For morphological descriptions, see the Identification/Diagnostic resources on the AMPS pest page on the CAPS Resource and Collaboration website.

Pathogen Description

Jujube Witches' Broom (JWB), a devastating disease of the jujube tree, is associated with the presence of '*Candidatus* Phytoplasma ziziphi', which belongs to the elm yellows (EY) group of phytoplasmas (subgroup 16SrV-B) (Gasparich et al., 2020). The reference strain of this phytoplasma, JWB-G1, is associated with the 16S rRNA gene sequence deposited in GenBank under the accession number AB052876 (Bertaccini et al., 2022). This phytoplasma is also the putative causal agent of diseases in stone fruit trees (Gao et al., 2020; Khan et al., 2013; Yue et al., 2009; Zhu et al., 1997), apple trees (Li et al., 2014b), and other plants (Li et al., 2009; Wang et al., 2015a; Wang et al., 2015b; Yang et al., 2011). Mixed infection with the 16SrI group of phytoplasmas within a single tree has been reported (Lee et al., 2009; Sun et al., 2013). '*Ca.* P. ziziphi' cells are approximately 200–500 nm in size and colonize the sieve tubes of infected trees’ phloem (Park et al., 2021; Zhao et al., 2019).

Symptoms

Plants infected by ‘*Ca.* P. ziziphi’ or related strains typically develop small leaves, phyllody (leaf-life structures developing in place of flower parts), virescence (abnormal greening), witches' broom (Fig. 1B), yellowing, and dwarfing symptoms (Chen et al., 2022; Zhao et al., 2019). Symptoms vary in different hosts.

Jujube:
Typical symptoms include witches' broom and proliferation of shoots and small leaves, dense root suckers and the absence of defoliation during winter, yellowing, stunted growth and plant decline (Fig. 1) (Chen et al., 2022), elongated peduncle, phyllody (Fig. 2 B,C) (Xue et al., 2020), deformed fruit or no fruit. Infected trees may die in 3 to 5 years (Chen et al., 2022; Zhao et al., 2019).

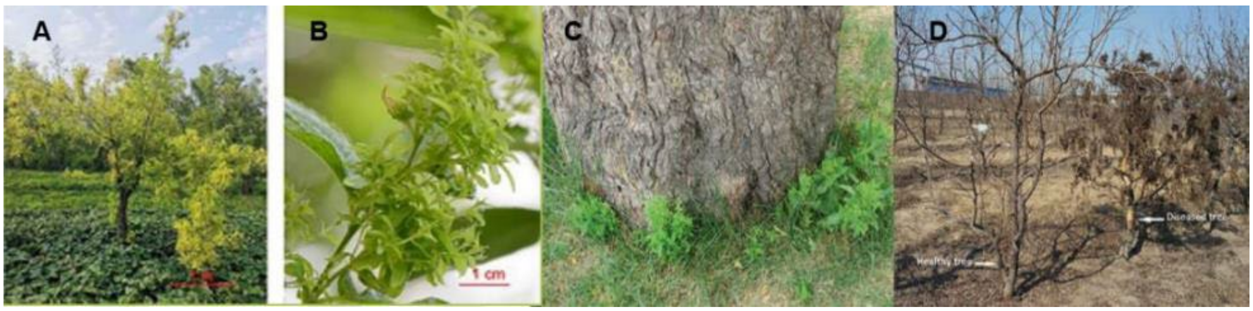

Figure 1. Symptoms due to '*Ca.* P. ziziphi' infection include: tree yellowing, stunting, and decline (A); Witches' broom of shoots (B); dense root suckers (C), lack of defoliation in the winter (D) on jujube. Photos A and B are from Chen et al. (Chen et al., 2022), used under the terms of the Creative Commons Attribution License; photos C and D are courtesy of Dr. Zhao (Agricultural University of Hebei) (Zhao et al., 2019).

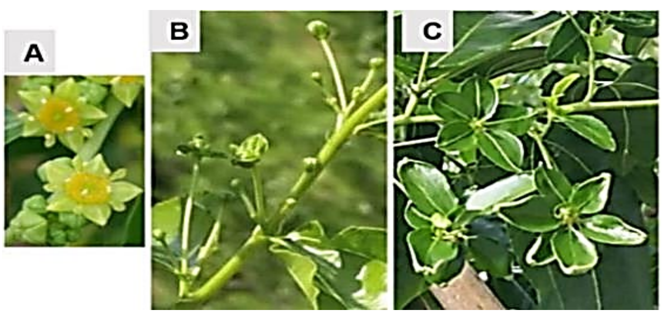

Figure 2. Healthy flowers (A) compared to '*Ca*. P. ziziphi' infected flowers with elongated peduncle (B) and phyllody flowers (C) in jujube tree. Photos courtesy of Dr. Zhao (Agricultural University of Hebei) (Xue et al., 2020).

Peach: Symptoms of '*Ca.* P. ziziphi' in peach include leaf scorch, leaf yellowing, leaf rolling, premature drying of fruit and fruit drop, and tree decline/death (Fig. 3) (Khan et al., 2013; Li et al., 2014a).

Figure 3. Symptoms of infected peach tree include yellow leaves (A), dieback (B), little and withered fruit (C), and necrotic spots on the leaves (D). Photos courtesy of Dr. Yunfeng Wu (Northwest A&F University) (Li et al., 2014a).

<u>Apricot:</u> The earliest visible symptom is upward-rolling leaves (Fig. 4C). In severely infected trees, the leaves show irregular yellowing with a tendency to curl upwards and shrink due to dehydration. Early fruit drop, reduced fruit set, delayed fruit maturity, and reduced fruit quality are also symptoms. Infected plants may wither and die after continuous infections (Fig. 4) (Han et al., 2021).

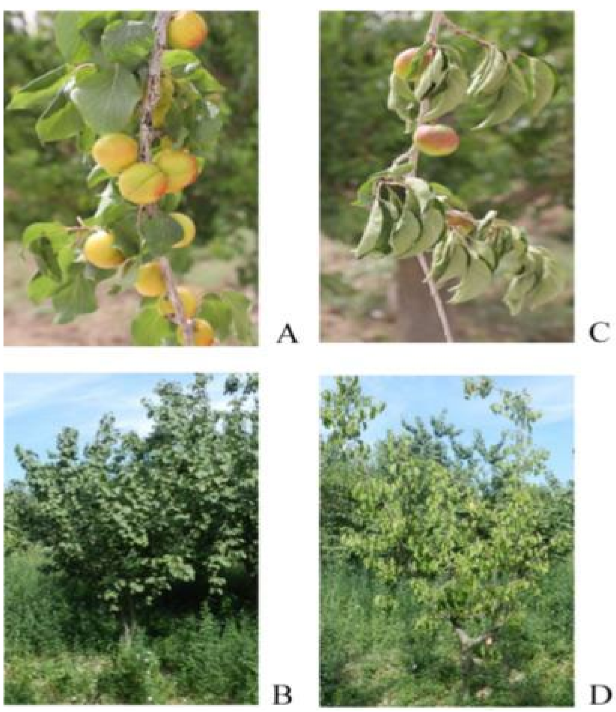

Figure 4. Fruits on a healthy fruit tree (A) compared to those on a '*Ca*. P. ziziphi' infected tree with reduced fruit set and delayed fruit maturity (C); a healthy tree (B) compared to a diseased tree with yellowing, upward-rolling leaves (D). Photos courtesy of Mr. Jian Han (Han et al., 2021)

<u>Apple</u>: Symptoms include yellowing, small leaves, and leaf margins rolling up (Li et al., 2014b).

Japanese plum: Symptoms of '*Ca.* P. ziziphi' infection in plum include little leaves, branches with shorten internodes, and witches' broom (Gao et al., 2020).

Persimmon: One to two-year old seedlings or new shoots are vulnerable to infection. Typical symptoms include stem fasciation (flattened stems), small leaves, and internode shortening with the clustering of leaves. Some infected branches can recover from the disease after about 3 to 4 years from the initial infection (Wang et al., 2017; Wang et al., 2015a).

Honey locust: Leaves on infected plants turn yellow. Infected branches may wither and die in later stages of infection (Du et al., 2013).

Cherry: Symptoms of '*Ca.* P. ziziphi' infection in cherry include small, yellowing, and curling leaves, witches' broom (Tan et al., 2015; Tan et al., 2017), floral virescence, failure of fruit to set, and branch death (Wang et al., 2018; Wang et al., 2014a).

Chinese cherry: Infected Chinese cherry produce abnormal, small fruits that fail to ripen (Li et al., 2022) and floral pats that develop into green leafy structures (Gao et al., 2019).

Easily Mistaken Species

Phytoplasma infection produces symptoms that may resemble those of other pathogens or abiotic stress and requires molecular and/or immunological confirmation. Other phytoplasmas present in the United States can infect the same hosts as '*Ca*. P. ziziphi' and be associated with similar symptoms. For example, flower virescence has been observed in sweet cherry associated with '*Ca*. P. ziziphi' (Wang et al., 2018; Wang et al., 2014a) and '*Ca.* P. asteris' (16SrI-S) (Gao et al., 2011). The etiological agent associated with elm yellows phytoplasma has been identified as '*Ca.* P. ziziphi' in China (Zhu et al., 2008) but as '*Ca*. P. ulmi' (16SrV-A) in Europe and the United States (Lee et al., 2004). Symptoms of X-disease (attributed to '*Ca*. P. pruni', 16SrIII-A infections) in stone fruits in North America are similar to those of '*Ca.* P. ziziphi' infection in peach (Khan et al., 2013; Li et al., 2014a; Uyemoto and Kirkpatrick, 2011). '*Ca.* P. pruni', '*Ca.* P. asteris' and '*Ca.* P. ulmi' are all present in the United States (Gasparich et al., 2020). Molecular analysis can differentiate these phytoplasmas (Lee et al., 1998; Lee et al., 2004; Zhao et al., 2009).

Biology and Ecology

The distribution of the phytoplasma in the tree is uneven and inconsistent throughout the year (Zhao et al., 2006). '*Ca.* P. ziziphi' overwinters in branches and roots (Yi et al., 2001; Zhao et al., 2006), where it can be detected year round using fluorescence microscopy or PCR techniques. Then, in the following spring, it migrates to newly emerged leaves and root suckers of the tree, where it can be detected 24 days or 12 days after leaves emerge, respectively. The phytoplasma usually is detected on the same side of infected branches and roots. '*Ca.* P. ziziphi' can tolerate low temperature of 5°F in aboveground parts during winter (Shi et al., 2014).

Hishimonus sellatus is the primary vector of '*Ca*. P. ziziphi' in China (Cui, 1991), Korea (La and Woo, 1980), and Japan (Kusunoki et al., 2002). *H. sellatus* can produce three (Sun et al., 1988) to four generations (Cui, 1991) per year in Hebei Province of China and overwinters as adults (third- or fourth- generation) on shelter plants, usually pine (*Pinus tabulaeformis*) or arborvitae (*Platyladus orientalis*) adjacent to jujube orchards. Occasionally, leafhopper eggs will overwinter beneath the bark of jujube branches.

When jujube buds begin to sprout in mid-April to early May, overwintered adults of *H. sellatus* return to jujube trees for oviposition. Peak egg laying occurs from mid-May to Mid-June. *H. sellatus* completes two or three full generations on jujube trees and adults of the third or fourth generations migrate back to shelter plants from late-August to late-October to overwinter (Cui, 1991; Sun et al., 1985). Adults of the first-, second-, and third- generation emerge at the end of May, early July, and Mid-August, respectively (Sun et al., 1988).

In addition to vector transmission, '*Ca.* P. ziziphi' is also commonly transmitted by grafting, propagation of root suckers, and dodder, a parasitic plant (Tian et al., 2002; Wu and Tian, 2019). However, it cannot be transmitted by soil, seed, pollen, or sap inoculation (Chen et al., 1984; La and Woo, 1980). In wild or uncultivated areas, *Taxillus chinensis* (mulberry mistletoe) (Xi et al., 2022), a parasitic plant, can also be a vector of '*Ca.* P. ziziphi'.

Although '*Ca.* P. ziziphi' was reported to infect peach (Khan et al., 2013) and jujube (Khan et al., 2008) in India, the vectors in that country are unknown.

Known Hosts

'*Ca.* P. ziziphi' is associated with serious damage on economically important jujube cultivars, as well as wild jujubes (Khan et al., 2008; Liu et al., 2004a; Zhao et al., 2019). An increasing number of fruits, horticultural plants, and forest species have been identified as natural hosts of '*Ca.* Phytoplasma ziziphi' with the aid of molecular technologies. Among them, *Euonymus bungeanus* (spindletree), *Hovenia dulcis* (Japanese raisin), *Hovenia tomentosa* (hovenia), *Prunus armeniaca* (apricot), and *Prunus persica* (peach) have been further verified by transmission experiments (Kamala-Kannan et al., 2011; Li et al., 2014a; Miyashita and Kusunoki, 2008; Ren et al., 2017). Host susceptibility varies among cultivars (Liu et al., 2004a; Zhao et al., 2019).

'*Ca.* P. ziziphi' can also be detected in hosts that exhibit no symptoms, such as *Amaranthus retroflexus* (common amaranth) growing around or under jujube trees (Yang et al., 2011). The knowledge of the biological and epidemiological characteristics of '*Ca.* P. ziziphi' in newly identified natural hosts is still limited.

The host list below includes cultivated and wild plants that 1) are infected or infested by the pest under natural conditions, 2) are frequently described as major, primary, or preferred hosts, and 3) have primary evidence for feeding and damage documented in *the literature. Plants are highlighted in bold if they are commercially produced and the pest causes economically significant damage.*

Major hosts: *Malus* spp. (apple)*, (Li et al., 2014b), ***Prunus armeniaca* (apricot)*** (Han et al., 2021), ***Prunus persica* (peach)*** (Khan et al., 2013), ***Ziziphus jujuba* (jujube)**[*] (Jung et al., 2003), *Ziziphus nummularia* (jujube) (Khan et al., 2008).

Other known hosts:
Amaranthus retroflexus (redroot amaranth)*, *Berberis thunbergii* (Japanese barberry)*, *Broussonetia papyrifera* (paper mulberry)*, *Camellia japonica* (Camellia)*, *Cannabis* spp. (hemp), *Cichorium intybus* (chicory)*, *Dianthus chinensis* (Chinese pink)*, *Diospyros kaki* (persimmon)*, *Euonymus bungeanus* (winterberry euonymus)*, *Hovenia dulcis* (Japanese raisintree)*, *Hovenia tomentosa* (hovenia), *Gleditsia sinensis* (Chinese honeylocust), ***Gleditsia triacanthos* (honey locust)***, *Impatiens balsamina* (spotted snapweed)*, ***Ipomoea batatas* (sweet potato)***, *Lactuca sativa* (garden lettuce)*, *Liriodendron chinensis* (Chinese tulip tree), ***Medicago sativa* (alfalfa)***, *Orychophragmus violaceus* (purple-Mistress/Chines violet)*, *Paliurus hemsleyanus* (Tong qian shu), *Phragmites australis* (common reed)*, ***Prunus avium* (sweet cherry)** *, *Prunus cerasifera* (cherry plum)*, *Prunus pseudocerasus* (Chinese cherry), ***Prunus salicina* (Japanese plum)***, ***Prunus serrulata* (flowering cherry)***, *Robinia pseudoacacia* (black locust)*, *Salix babylonica* (weeping willow)*, *Senna surattensis* (glossy shower/sunshine tree)*, *Sophora alopecuroides* (Ku dou zi), *Spiraea salicifolia* (willow-leaf meadowsweet)*, *Styphnolobium japonicum* (syn. *Sophora japonica*) (Japanese pagoda tree) *, *Syringa reticulata* (Japanese tree lilac)*, *Taxillus chinensis* (mulberry mistletoe), *Trifolium repens* (white clover)*,*Trifolium pratense* (red clover)*, and *Ulmus parvifolia* (Chinese elm)*.(Du et al., 2013; Gao et al., 2019; Gao et al., 2020; Gao et al., 2015; Han et al., 2021; Hong et al., 2011; Kamala-Kannan et al., 2011; Khan et al., 2013; Kusunoki et al., 1994; Lai et al., 2022; Li et al., 2013a; Li et al., 2013b; Li et al., 2021; Li et al., 2011; Li et al., 2012a; Li et al., 2012b; Li et al., 2010a; Li et al., 2009; Li et al., 2014b; Li et al., 2010b; Li et al., 2012c; Li et al., 2013c; Liu et al., 2004b; Min et al., 2009; Miyashita and Kusunoki, 2008; Ren et al., 2014; Ren et al., 2017; Shi et al., 2013; Wang et al., 2015a; Wang et al., 2014a; Wang et al., 2014b; Wang, 2015; Wang et al., 2015b; Wu et al., 2012; Yang et al., 2020; Yang et al., 2011; Yu et al., 2012; Yue et al., 2009; Zhang et al., 2010; Zhao et al., 2007; Zhu et al., 2008).

‘*Ca.* P. ziziphi’ can infect crops that are intercropped with jujube tree, such as amaranth, alfalfa, and sweet potato, either symptomatically or asymptomatically (Li et al., 2021; Li et al., 2012b; Yang et al., 2011). Those crops can be reservoir hosts of the phytoplasma.

Broussonetia papyrifera (paper mulberry) is also a favorable host and preferred oviposition sites of the insect vector *Hishimonus sellatus* (Cui, 1991).

Reported hosts with uncertainties:

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[*] Hosts with known U.S. distribution

Solanum tuberosum (potato) was reported as a host of '*Ca*. P. ziziphi' but the 16SrRNA gene sequence isolated from infected plants only shared 97.51% similarity with the16SrV-B group (Sagar et al., 2020), which means it can be considered a new species based on the available guidelines (Bertaccini et al., 2022).

Ligustrum japonicum (Japanese privet)*, *L. foliosum* (Korean privet), *L. lucidum* (glossy privet)*, *L. obtusifolium* (border privet)*, and *Syringa vulgaris* (lilac)* are listed as hosts of '*Ca.* P. ziziphi ' (Cho and Shin, 2004; Jung et al., 2012), but no primary resources can be found.

Pest Importance

'*Ca*. P. ziziphi' is one of the most destructive pathogens of jujube in China, Korea, India, and Japan (Khan et al., 2008; Kusunoki et al., 2002; La and Woo, 1980; Zhao et al., 2019). In severe outbreaks, this pathogen caused 30 to 80% economic loss due to reduction in quantity and quality of fruit (Zhao et al., 2019). Infected trees often die within 3 to 4 years following the onset of symptoms (Kusunoki et al., 2002). Young seedlings of susceptible cultivars may die in the same year and young trees can die in 1-2 years after infection (Tian et al., 2002). Jujube is commonly grown as an ornamental plant in the United States (Yao, 2013).

'*Ca*. P. ziziphi' is associated with economic damage to stone and pome fruit trees. In China, incidence of '*Ca*. P. ziziphi' in apricot was reported to be more than 30%, yield losses ranged from 20 to 50%, and trees died quickly after infection (Han et al., 2021). Infected sweet cherry trees wilted about one month after showing symptoms of virescence and eventually died; infected flowers in China did not produce fruit (Wang et al., 2014a). In a study from India, about 70% of tested peach trees were infected by '*Ca*. P. ziziphi' (Thakur et al., 1998). Peach infection has also been recorded in China and Italy, where infected trees declined and died, and the infected peach fruits shriveled up and dropped early (Khan et al., 2013; Li et al., 2014a; Paltrinieri et al., 2006; Thakur et al., 1998). In a study from China, about 3% of young apple trees were infected by '*Ca*. P. ziziphi' in China (Li et al., 2014b). Apple, apricot, cherry, peach and nectarine, and plum are all important fruit crops in the United States. In 2021, fresh apples were worth over $3 billion, sweet cherries were valued at $866 million, peaches and nectarine were valued at $759 million, fresh plums were valued at over $90 million, and apricots were valued at $37 million (USDA-NASS, 2022).

Other valuable hosts of '*Ca*. P. ziziphi' include alfalfa (Li et al., 2012b) and several ornamental or shade trees. Alfalfa is an important forage and cover crop and was planted on over 15 million acres in the United States in 2021, where it had an estimated harvest value $9.6 billion (USDA-NASS, 2022). Infected Japanese plum (Gao et al., 2020), flowering cherry (Wang et al., 2014b), honey locust (Du et al., 2013), and willow trees (Lai et al., 2022) have all been found in Chinese surveys. Flowering cherry (*Prunus serrulate*) is an ornamental tree in the United States (Ma et al., 2009), with sales reaching $33.9 million per year across 44 U.S. states; Honey locust and willow are popular deciduous shade trees in the United States with a value of $15.6 and $11.2 million, respectively per year (USDA-NASS, 2022).

Sweet potatoes, which can act as a reservoir host of this pathogen, were often found to be infected by ‘*Ca*. P. ziziphi’ when cultivated under jujube trees in China (Li et al., 2021). While there is no evidence of yield loss, sweet potatoes potentially at risk and are a major crop in the United States with a total value of $680 million.

‘*Ca.* P. ziziphi’ is listed as a harmful organism in European Union (APHIS, 2022). There may be trade implications with these countries if this pest becomes established in the United States.

Pathogens or Associated Organisms Vectored

This species is not known to vector any pathogens or associated organisms, but damage may lead to invasion by secondary pests.

Known Insect Vectors (or putative insect vectors)

Hishimonus sellatus Uhler (Fig. 5) is a confirmed major vector of '*Ca*. P. ziziphi' in China, Japan, and Korea (Kusunoki et al., 2002; La and Woo, 1980; Sun et al., 1988). *Hishimonoides chinese, Hishimonoides aurifacialis,* and *Typhlocyba* sp. have been documented as vectors of this disease on jujube tree in China (Chen et al., 1984; Sun, 1990; Sun et al., 1985; Wang et al., 1981). *Hishimonus sellatus* can transmit several phytoplasmas in different plant host species (Kusunoki et al., 2002). *Hishimonus sellatus* was collected in the United States on the Boston Harbor Islands, and there are unconfirmed reports of this insect on the Massachusetts mainland (Rykken and Albert, 2012).

Besides China, India, Japan, and Korea, where *'Ca.* P. ziziphi' has been recorded, *Hishimonus sellatus* is also present in Afghanistan, Armenia, Australia, Ethiopia, Georgia, Indonesia, Malaysia, Papua New Guinea, Philippines, Russia, Sri Lanka, and Tanzania (Du and Dai, 2019). *Hishimonoides chinese* and *Hishimonoides aurifacialis* are only present in China (Dai et al., 2010).

Figure 5. *Hishimonus sellatus* Uhler, a major vector of ‘*Ca.* P. ziziphi’, Photo courtesy of Prof. Wang (Beijing University of Agriculture) (Hao et al., 2015).

'*Ca*. P. ziziphi' has been detected by PCR in *Hishimonus lamellatus,* which occurs with *H. sellatus* in jujube orchards, but the vector status of this insect has not been confirmed (Hao et al., 2015).

Known Distribution

Asia: China (Zhu et al., 1997), Japan (Maejima et al., 2014), India (Khan et al., 2008), South Korea (Jung et al., 2012; Kamala-Kannan et al., 2011),

Europe: Italy (Paltrinieri et al., 2008; Paltrinieri et al., 2006; Paltrinieri et al., 2005)

In Italy, ‘*Ca*. P. ziziphi’ was found in mixed infections with ‘*Ca*. P. solani’ and ‘*Ca*. P. asteris’ (Paltrinieri et al., 2008; Paltrinieri et al., 2006). The known vectors of this phytoplasma are not present in Italy where studies on its insect vectors were not carried out.

Pathway

Long-distance spread of ‘*Ca*. P. ziziphi’ occurs when infected plant material, especially asymptomatic plant material, is transported to a new area (Tian et al., 2002). In jujube, transport of infected root suckers for propagation or infected seedlings were considered as the primary means of introduction of ‘*Ca.* P. ziziphi’ into new production areas (Li et al., 2019; Tian et al., 2002). Local spread of ‘*Ca.* P. ziziphi’ may occur via the movement of infected insect vectors.

According to Federal Order DA-2013-18, effective May 20, 2013, the import of *Malus* spp. and *Prunus* spp. propagative material is currently restricted to prevent the spread of *Anoplophora chinensis* (Chinese longhorned beetle, CLB) and *Anoplophora glabripennsis* (Asian longhorned beetle, ALB). Currently, *Prunus* spp. may be imported from Canada and the Netherlands, and *Malus* spp. may be imported from Belgium, Canada, France, Germany, and the Netherlands. Propagative material grown in Canada is not regulated for CLB or ALB and does not have specific import requirements, but propagative material from other approved countries (Belgium, France, Germany, and the Netherlands) are required to be declared free from pathogens and are subjected to post-entry quarantine following import.

Use the PPQ Commodity Import and Export manuals listed below to determine 1) if host plants or material are allowed to enter the United States from countries where the organism is present and 2) what phytosanitary measures (e.g., inspections, phytosanitary certificates, post entry quarantines, mandatory treatments) are in use. These manuals are updated regularly.

Agricultural Commodity Import Requirements (ACIR) database: ACIR provides a single source to search for and retrieve entry requirements for imported commodities. https://acir.aphis.usda.gov/s/

Plants for Planting Manual: This manual is a resource for regulating imported plants or plant parts for propagation, including buds, bulbs, corms, cuttings, layers, pollen, scions, seeds, tissue, tubers, and like structures.
https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/plants_for_planting.pdf

Treatment Manual: This manual provides information about treatments applied to imported and domestic commodities to limit the movement of agricultural pests into or within the United States.
https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/treatment.pdf

Potential Distribution within the United States

Based on the known distribution of '*Ca*. P. ziziphi', it may establish in Plant Hardiness Zones 4-11, which encompass most of the continental United States (Takeuchi et al., 2018). Commercial orchards of apple, apricot, cherry, peach, and plum are the most vulnerable to infection and spread of '*Ca*. P. ziziphi'. The top stone fruit producing states in 2021 were California, Washington, South Carolina, Oregon, and Georgia. California accounted for about 60% of all U.S. stone fruit production (USDA-NASS, 2022). The top apple producing states in 2021 were Washington, New York, Michigan, Pennsylvania, California, Virginia, and Oregon. Washington accounted for about 70% of all U.S. apple production (USDA-NASS, 2022). Jujube, the main host of '*Ca*. P. ziziphi', is adapted and grows well in the Southern and Southwestern United States (Yao, 2013).

Survey and Key Diagnostics

Approved Methods for Pest Surveillance:

For the current approved methods and guidance for survey and identification, see Approved Methods for Pest Surveillance (AMPS) pest page on the CAPS Resource and Collaboration website, at https://caps.ceris.purdue.edu/approved-methods.

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Versions

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