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National Plant Disease Recovery System

Recovery Plan for X-Disease in Stone Fruit Caused by '*Candidatus* Phytoplasma pruni'

Scott J. Harper,[1,†] Tobin D. Northfield,[2] Louis R. Nottingham,[3] S. Tianna DuPont,[4] Ashley A. Thompson,[5] Bernardita V. Sallato,[6] Corina F. Serban,[6] Madalyn K. Shires,[7] Alice A. Wright,[8] Katlyn A. Catron,[2] Adrian T. Marshall,[2,9] Cody Molnar,[1] and W. Rodney Cooper[9]

[1] Department of Plant Pathology, Washington State University, Prosser, WA

[2] Department of Entomology, Washington State University, Wenatchee, WA

[3] Department of Entomology, Washington State University, Mt. Vernon, WA

[4] Tree Fruit Extension, Washington State University, Wenatchee, WA

[5] Fruit Tree Extension Wasco and Hood River Counties, Oregon State University, The Dalles, OR

[6] Tree Fruit Extension, Washington State University, Prosser, WA

[7] Department of Agronomy, Horticulture & Plant Science, South Dakota State University, Brookings, SD

[8] Sugarcane Research Unit, U.S. Department of Agriculture – Agricultural Research Service, Houma, LA

[9] Temperate Tree Fruit and Vegetable Research Unit, U.S. Department of Agriculture – Agricultural Research Service, Wapato, WA

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[†] Corresponding author: S. J. Harper; scott.harper@wsu.edu

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Stone fruits are a multibillion-dollar industry for the United States and Canada, one that has repeatedly suffered significant economic losses due to outbreaks of the X-disease phytoplasma ('*Candidatus* Phytoplasma pruni') over the last century. Orchards and entire production areas have been abandoned, with corresponding losses to growers, fruit packers, and consumers. The most recent outbreak, in the U.S. Pacific Northwest, resulted in an estimated $65 million (USD) loss in revenue between 2015 and 2020 and is only increasing in incidence. Already present across much of the continental United States and Canada, the phytoplasma has a broad host range beyond stone fruit and is transmitted by at least eight leafhopper species, therefore stone fruit production in every state is at significant risk. This recovery plan was produced as part of the National Plant Disease Recovery System and is intended to provide a review of pathogen biology, assess the status of critical recovery components, and identify disease management research, extension, and education needs.

Executive Summary

Stone fruits are a multibillion-dollar industry for the United States and Canada, with sweet cherry, sour cherry, peach, nectarine, and plum production spread across much of continental North America. This industry is threatened by a range of bacterial, fungal, and viral pathogens, one of the most significant of which is '*Candidatus* Phytoplasma pruni', the causal agent of X-disease. Recurring epidemics of this pathogen have, for the last century, caused major economic harm through lost yield, trees dead or removed by growers, and the temporary or permanent abandonment of growing areas.

In stone fruit, the primary effect of this pathogen is to disrupt normal fruit development, leading to fruit that are immature and smaller, paler, more distorted, and with poorer flavor when compared to fruit from uninfected trees. It also causes, depending on the *Prunus* species infected, foliar chlorosis, decline and dieback of individual limbs, and, in some cases, death of the tree. Infected trees cannot be treated or cured, and the only effective management strategy is to identify and remove infected trees and apply insecticides to suppress vector populations.

This pathogen is epidemiologically complex and is reportedly transmitted by at least eight species of leafhoppers (Hempitera: Cicadellidae: Deltocephalinae) in addition to being graft transmissible and spread through propagation practices. It can infect nearly all *Prunus* species and interspecific hybrids, as well as a broad range of annual, biennial, and perennial plants including dandelion, mallow, apple, and sagebrush. These factors contribute to its distribution and persistence; the X-disease-causing and related strains of '*Ca*. P. pruni' are present across much of the United States and Canada. Outbreaks are cyclical, with a slow buildup followed by a state or regional epidemic that can take over a decade of active management to suppress.

With an average of 30 years between outbreaks, research on all aspects of the pathosystem, including the biology of the pathogen itself, disease expression, and host range, has been sporadic, as has research into the leafhoppers that vector the pathogen. Absent an outbreak, research efforts have been focused elsewhere. This also means that X-disease research has not benefited from advancements in molecular biology and our understanding of this pathogen lags behind other important systemic plant pathogens. Therefore, when an outbreak such as the current epidemic in the United States Pacific Northwest occurs, much basic and applied research needs to be performed to successfully develop an effective area-wide management program. Core areas of research, extension, and education include:

- Characterization of the biology of the X-disease phytoplasma, including pathogen–host and pathogen–vector interactions and disease expression.
- Improved understanding of the phytoplasma–vector–host paradigm and identifying factors of biological significance.

- Development of methods, approaches, and policies to suppress or prevent pathogen and vector spread.
- Continued education of stone fruit industry members on the importance and economic impacts of X-disease in their orchards, and on pathogen and vector biology.
- Promotion of local and regional pathogen, host, and vector management and recovery programs, including quarantines and enforcement necessary to protect orchards and the supply of propagative material from nurseries.

I. Introduction

'*Candidatus* Phytoplasma pruni' is the causal agent of X-disease, a debilitating disorder affecting species within the genus *Prunus* for which it induces a reduction in fruit development, as well as foliar chlorosis or anthocyanosis, premature leaf drop, and decline and dieback. This pathogen has caused significant economic damage for over a century, causing crop loss and orchard removal in outbreaks in both the eastern and western United States and Canada (Blake et al. 1921; Palmiter and Hildebrand 1943; Purcell et al. 1987; Rawlins and Thomas 1941; Smith 1894; Wright et al. 2021b).

The story of '*Ca*. P. pruni' and United States fruit production begins with reports of "peach yellows" and "little peach" in Pennsylvania, New Jersey, Delaware, Maryland, and Connecticut in the early 19th century (Blake et al. 1921). As the names suggest, leaves were yellow with red spotting and drooped and rolled inwards, and fruit were smaller, with blotchy skin color, and distorted (Blake et al. 1921; Cook 1921; Smith 1894). A series of more serious and damaging outbreaks followed from the 1870s onwards across much of the northeast and Midwest (Table 1), which were brought under control by extensive tree removal programs in the 1890s (Smith 1894), such that by the 1920s, "peach yellows" and "little peach" were considered successfully suppressed (Adams 1923).

The first reports of what came to be known as X-disease date from the early 1930s, with outbreaks of fruit- and leaf-affecting diseases in cherry and peach across much of the United States (Table 1). The first of these was "buckskin" in California, which was first observed around 1927, where cherry fruit were smaller than normal, pointed, and failed to develop the expected coloration, remaining a dull red, pale pink, or yellow depending on the variety (Rawlins and Horne 1931). Also in California, in 1932 peaches were described as showing "leaf casting yellows," foliar chlorosis and distortion with necrotic holes developing in the leaf blade, followed by premature leaf drop (Thomas et al. 1940). Trees were found to grow poorly early in the season, and fruit size was reduced with mummification and drop being common. At the same time in Connecticut, peaches were found to exhibit very similar symptoms, with the fruit further described as prematurely ripened and bitter; it is from this outbreak that the name "X-disease" originated, appropriating the mathematical term "X" as the disease was an unknown quantity (Stoddard 1938).

As word of these diseases spread, researchers across the country began surveying (Table 1) or in some cases associated previously observed symptoms with these new diseases (Gilmer and Blodgett 1976). For example, a survey in New York in 1938 found widespread incidence of "yellow-red virosis" on peach and chokecherry, the two colors in the name representative of the foliar symptoms observed on these two hosts (Palmiter and Hildebrand 1943); similar surveys found X-disease in Illinois in 1939 (Seifert and Anderson 1939), in Michigan and Wisconsin in 1941 (Cation 1941; Dhanvantari and Kappel 1978), and in Pennsylvania and Ohio in 1944 (Harris 1944; Zundel 1944). Further west, X-disease was reported in Utah in 1937 and associated with "red leaf" in chokecherry and "wilt and decline" of sweet and sour cherries on mahaleb rootstock (Richards and Cochran 1956), and was also found in Colorado in 1941 (Bodine and

Durrell 1941). Finally, in the Pacific Northwest, X-disease was identified in 1935, 1936, and 1939 for Washington, Idaho, and Oregon, respectively (Blodgett 1939; Richards and Cochran 1956; Zeller and Evans 1941). Interestingly, two other diseases, "cherry albino" and "Montmorency pink fruit," were described from Oregon and Washington, respectively, in the 1930s (Cameron 1976; Cheney et al. 1976), with atypical X-disease-like symptoms; these may represent local strains or variants of the phytoplasma.

In Canada, X-disease was found in Ontario in 1941 (Dhanvantari and Kappel 1978), the timing corresponding with outbreaks in U.S. border states. A disease termed "small bitter cherry", reported in British Columbia in 1937 (Foster and Lott 1947; Lott 1959), may have been little cherry virus 2 (LChV2); while LChV2 was later attributed to be the primary agent in the British Columbia epidemic of the 1930s onwards (Wilks and Milbrath 1956), the presence of X-disease in nearby Washington state suggests that both pathogens could have been present.

After the initial X-disease epidemic across much of North America in the 1930s through the 1940s, nearly 30 years elapsed before the next major outbreaks, in California (Purcell et al. 1987), Michigan, and Ontario (Dhanvantari and Kappel 1978) in the 1970s. Extensive tree removal again successfully suppressed the disease (Purcell et al. 1987), and another 30 years elapsed before a small number of positives were found in Pennsylvania (Nikolaeva et al. 2017) and Washington beginning in 2010 (Villamor

TABLE 1
Reports of X-disease or related syndrome outbreaks in the United States and Canada[a]

Country	State/province	X-disease first reported	Later X-disease outbreaks	Peach yellows and little peach	Reference
U.S.A.	Arizona	1952			Kenner (1953)
	California	1931	1970s–1980s, 2003–2008, 2019		Purcell et al. (1987); Rawlins and Horne (1931); M. Nouri, *personal communication*
	Colorado	1941			Bodine and Durrell (1941)
	Connecticut	1933		1810*	Blake et al. (1921); Stoddard (1938)
	Delaware			1887	Blake et al. (1921)
	Idaho	1936	2019		Blodgett (1939); Harper, *unpublished data*
	Illinois	1939		1890s*	Siefert and Anderson (1940); Smith (1894)
	Indiana			1890s*	Smith (1894)
	Maryland			1890s*	Smith (1894)
	Massachusetts	1934		1890s*	Blake et al. (1921); Boyd (1938)
	Michigan	1941	1970s*	1879	Blake et al. (1921); Cation (1941); Dhanvantari and Kappel (1978)
	New Hampshire	1940*			Stevens and Stevens (1941)
	New Jersey			1890s*	Smith (1894)
	New York	1938		1890s*	Palmiter and Hildebrand (1943); Smith (1894)
	North Dakota	1996			Guo et al. (1996)
	Ohio	1944		1890s*	Harris (1944); Smith (1894)
	Oregon	1939	2020–present		Reinhold and Pscheidt (2022); Zeller and Evans (1941)
	Pennsylvania	1944	2013	1800s*	Blake et al. (1921); Nikolaeva et al. (2016); Zundel (1944)
	Utah	1937	2019		Richards and Cochran (1956); B. Black, *personal communication*
	Vermont	1940*			Stevens and Stevens (1941)
	Virginia			1890s*	Smith (1894)
	Washington	1935	2010–present		Richards and Cochran (1956), Wright et al. (2022)
	Wisconsin	1941			Dhanvantari and Kappel (1978)
Canada	Ontario	1941	1970s*		Dhanvantari and Kappel (1978)
	New Brunswick	1964			Callahan (1964)
	British Columbia	1937*			Foster and Lott (1947); Lott (1947)
	Alberta	1994			Hiruki and Wang (1999)

[a] Dates marked with an asterisk are approximate due to the primary sources' uncertainty regarding when the outbreak began in the state or province.

and Eastwell 2019). Initially thought to be an outbreak of LChV2, X-disease rapidly overtook the virus as the primary pathogen in the Pacific Northwest, causing significant crop loss (Wright et al. 2021b). At the time of writing, the X-disease epidemic continues in the Pacific Northwest.

X-disease was originally thought to be caused by a virus based on its graft and vector transmissibility and inability to be cultured (Rawlins and Thomas 1941; Richards and Cochran 1956; Stoddard 1938). The pathogen was later identified as a mycoplasma-like organism by electron microscopy with the observation of pleomorphic cells of 200 to 400 nm in both infected leafhoppers and celery, a herbaceous host of the pathogen (Nasu et al. 1970). Hybridization with DNA probes indicated that this was a species distinct from other phytoplasmas identified at the time (Jiang et al. 1989; Lee et al. 1992). This was supported by restriction fragment length polymorphism and sequence analysis of the X-disease phytoplasma's 16S ribosomal RNA gene in which it was classified as part of the 16SrIII-A group (Lee et al. 1993, 1998). The species '*Candidatus* Phytoplasma pruni' was proposed in 2004, and formally adopted as a species in 2013 to encompass the 16SrIII-A phytoplasmas including X-disease, peach yellows, little peach, peach rosette, and red suture isolates (Davis et al. 2013).

This phytoplasma is transmitted by propagation and grafting of infected material, and by several different leafhopper species. The primary vectors change with the growing region and, potentially, the '*Ca*. P. pruni' strains present (Stoddard 1947). For example, *Colladonus geminatus* is the primary vector of X-disease in Utah (Richards and Cochran 1956), while *Paraphlepsius irroratus* is the most significant vector in Michigan (Rosenberger and Jones 1978). Interestingly, "peach yellows" and "little peach" were found to be vectored by *Macropsis trimaculata* (Kunkel 1933) but this leafhopper was not found to transmit isolates associated with X-disease (Stoddard 1947). This would suggest a close strain–vector relationship that may explain differential patterns of emergence and spread across the country.

Reports of '*Ca*. P. pruni' in the last century and across the country with different patterns of transmission, pathogenicity, and virulence (Jensen 1956; Palmiter and Hildebrand 1943; Rawlins and Horne 1931; Rawlins and Thomas 1941; Richards and Cochran 1956; Wright et al. 2021b) are indicative of the existence of different strains of this phytoplasma. For example, during the 1930s outbreak in California, three strains, 'Green Valley', 'Napa', and 'Siebe', were described, each with different pathogenicity on sweet cherry (Jensen 1956; Rawlins and Thomas 1941), while in New York, differential reactions from what were proposed as three different strains were described on chokecherry (Gilmer et al. 1954; Palmiter and Hildebrand 1943). It is unlikely, given the distance and limited movement in propagative material at the time, that these were the same three strains in two distinct locations. Instead, it is likely that '*Ca*. P. pruni' strains have evolved and diversified over time and that there will be continued, changing patterns of emergence and spread. The current epidemic in the Pacific Northwest may be indicative of that, where early multilocus characterization suggested the presence of two strains, one of which was similar to 'Green Valley' and the other different (Villamor and Eastwell 2019), while more recent work based on partial genomic sequences suggests there may be several more strains present in the region (Harper, *unpublished data*).

Finally, options for control of '*Ca*. P. pruni' in *Prunus* are limited. Removal of infected trees has been the most effective approach for the last century (Blake et al. 1921; Palmiter and Hildebrand 1943; Purcell et al. 1987; Richards and Cochran 1956; Van Steenwyk et al. 1995), albeit one that many cherry and peach growers are loath to perform. Control of vectors is also important in suppressing emergence and spread as demonstrated in California (Purcell et al. 1987) and in Ontario and Michigan in the 1970s where the removal of DDT as an option was associated with a resurgence in X-disease incidence (Dhanvantari and Kappel 1978). Finally, the management of nursery

systems to ensure that infected material is not being propagated, sold, and planted is essential (Richards and Cochran 1956); in the Pacific Northwest current infection rates of 5 to 12% have been found in new planting stock (Harper, *unpublished data*).

II. Disease Cycle and Symptom Development

The infection cycle of '*Ca*. P. pruni' in *Prunus* spp. begins with the inoculation of the pathogen into an uninfected host plant. This occurs naturally through leafhopper vector-mediated transmission from another infected host, with the leafhopper feeding on phloem bundles in leaf stems and midribs, secondary veins, and succulent terminal growth of the receptor tree. The length of feeding in natural conditions is unknown; experimental feeding lengths of hours to several weeks have been attempted (Gold and Sylvester 1982; Wolfe et al. 1951b). How much phytoplasma is egested during each feeding is similarly unknown and can only be estimated to be between tens to hundreds of cells based on phytoplasma titer determined by qPCR in severed heads and salivary glands of '*Ca*. P. pruni'-positive *Colladonus montanus* subsp. *reductus* and *Euscelidius variegatus* (Northfield et al., *unpublished data*). Infection of a tree may also occur by or through root grafting from an infected neighboring tree, or through human-mediated means by the unintentional grafting of infected material. In both cases this may introduce orders of magnitude more phytoplasma into the inoculated host than a leafhopper can, leading to more rapid disease onset (Harper et al., *unpublished data*).

Once it has been introduced into the host's phloem, phytoplasma movement away from the inoculation site is largely determined by the source-to-sink osmotic flow of photoassimilates within the tree's vascular system (Schaper and Seemüller 1982). Movement of the phytoplasma is generally basipetal towards the roots, although it may be transported to a local sink (Wright et al. 2022b) near the inoculation site depending on the strength of the sink pressure (Falchi et al. 2020). The flow rate and thus rate of movement of the phytoplasma changes with the seasonal development of the plant, with low levels of translocation during dormancy, and more rapid movement during the active growing season in spring and summer, slowing in fall as the tree enters dormancy (Ray and Savage 2021). The rate of movement is influenced by the plant tissue being transited, with lower rates of movement (18 to 42 cm h^{-1}) in leaves and higher rates (up to 100 cm h^{-1}) in stem and trunk tissues (Hidaka et al. 2019; Lalonde et al. 1999).

After translocation to and accumulation in the roots following initial inoculation, the phytoplasma gradually colonizes the tree systemically from the trunk upwards, accumulating in lower branches first, then aerial scaffolds and limbs (Wright et al. 2022b). A complete systemic infection of an orchard tree can take several growing seasons after infection, for while '*Ca*. P. pruni' does not die off completely in aerial limbs during winter dormancy, titer does drop by three to four orders of magnitude (Wright et al. 2022b) and reaccumulates the following spring. Infection also does not proceed uniformly within a tree, often one scaffold or side of the tree may become systemically infected before the remainder of the tree (Richards and Cochran 1956; Wright et al. 2022b).

Within the growing season, '*Ca*. P. pruni' accumulates in flower buds at bloom, increasing in titer as the ovary develops into fruit during the shuckfall and pit hardening stages, and through to harvest (Wright et al. 2022b). This localized accumulation is essential for disease expression for it has been observed that symptom onset and severity is titer-dependent (Wright et al. 2021b, 2022a). Given that the phytoplasmas are temperature sensitive, cold spring temperatures can suppress disease expression, leading to milder symptoms even in heavily infected trees; similarly, early flowering cherry varieties (Radičević et al. 2011) such as 'Chelan' appear to evade optimal timing of phytoplasma accumulation and therefore have slightly milder symptoms (Harper, *unpublished observations*).

Accumulation in leaves occurs out of cycle with accumulation in fruit (Wright et al. 2022b) due to the competition with shoot apices and developing fruit as sink tissues (Falchi et al. 2020). Titer in leaves is lower than in fruit until pit hardening, at which time it accumulates steadily until the post-harvest period and into predormancy (Wright et al. 2022b). This is likely why foliar symptoms, when expressed in *Prunus* species, occur in the summer and fall (Palmiter and Hildebrand 1943; Rawlins and Horne 1931; Rawlins and Thomas 1941; Richards and Cochran 1956; Wilks and Milbrath 1956; Wright et al. 2022a, b). The rapidity with which different *Prunus* species develop symptoms and the type and severity of symptoms presented depends on the infecting X-disease phytoplasma genotype, the species or cultivar infected, the phytoplasma titer, and how far the infection has progressed (Wright et al. 2021b, 2022a, b). Examples are presented below.

Symptoms on sweet and sour cherries. The most characteristic and well-described disease syndrome of '*Ca*. P. Pruni' in *Prunus* spp. is the production of small, underdeveloped, pale, and distorted sweet (*P. avium*) and sour (*P. cerasus*) cherries that are subjectively either bitter or tasteless (Purcell et al. 1987; Rawlins and Horne 1931; Rawlins and Parker 1933; Wilks and Milbrath 1956; Wright et al. 2021b). In these species, '*Ca*. P. pruni' infection is a long-term deliberative disease characterized by the gradual reduction in cherry fruit appearance and quality, with strain-dependent foliar and tree decline symptoms. Symptoms first appear on a single branch or leader, often only on a single cluster of fruit low on the tree, one to two years after infection, and over subsequent seasons symptom expression spreads systemically across the entire tree and increases in severity as the phytoplasma increases in titer (Rawlins and Horne 1931; Rawlins and Thomas 1941; Wright et al. 2021b, 2022b).

The most economically important symptom of '*Ca*. P. pruni' infection of both sweet and sour cherry is expressed on the fruit, in which the rate of fruit development is either reduced or halted from approximately the pit hardening or straw phases, although the difference between symptomatic fruit and asymptomatic fruit is often not clear until 2 weeks prior to harvest, depending on the variety infected (Wright et al. 2021b). In both species, fruit are smaller than normal, with a reduction of up to 50% in diameter and mass (Wilks and Milbrath 1956; Wright et al. 2021b), although this effect is cultivar-specific with lesser reductions in size observed in *P. avium* cultivars Bing, Santina, and Benton than in Rainier (Wright et al. 2021b). Similarly, fruit may be distorted, becoming lumpy, flattened, or have pointed tips on a cultivar- or strain-specific basis (Gilmer and Blodgett 1976; Rawlins and Horne 1931; Wright et al. 2021b), and when dissected, the mesocarp will be thinner, fibrous, or underdeveloped with smaller, often nonviable seeds (Shires et al., *unpublished data*). Finally, pedicels may be shorter and thicker depending on the infecting strain (Rawlins and Thomas 1941).

Fruit color development is similarly reduced, although the degree of reduction depends on both the infecting X-disease phytoplasma strain (Rawlins and Thomas 1941), cherry cultivar, and the titer of the phytoplasma in the infected branches (Wright et al. 2021b). Low-titer infections of dark sweet cherries result in bright or medium-red coloration rather than desired burgundy to black color, depending on the cultivar, whereas high-titer infections result in a more severe reduction, with fruit appearing pink, yellow, or white, or even green at harvest (Wright et al. 2021b). Yellow or blush cherries such as Rainier will have reduced blush coloration at low phytoplasma concentrations, and remain solid yellow, white, or green with high-titer '*Ca*. P. pruni' infections (Fig. 1A) (Wright et al. 2021b). In both types of cherry, color development may not be evenly reduced on individual fruit, with an uneven, blotchy, or dull appearance, which is the classic "buckskin" symptom first described in California during the 1930s (Rawlins and Horne 1931) and was proposed to be strain specific (Rawlins and Thomas 1941). Symptom expression is also influenced by growing location and environmental conditions (Wright et al. 2021b). For example, in 2022 the cold spring in central Washington state suppressed phytoplasma accumulation and fruit

symptom expression throughout the season with even heavily infected trees failing to express severe symptoms despite producing clear symptoms in the years prior (Harper, *unpublished data*).

Finally, the flavor of fruit is negatively impacted by '*Ca*. P. pruni' infection, and, while subjective, has been variously described as flavorless, bland, or bitter (Wilks and Milbrath 1956; Wright et al. 2021b). Mildly symptomatic fruit may have small, statistically insignificant, reductions in fructose, glucose, and sorbitol content that may be noticed when consumed (Wright et al. 2021b), whereas in severely symptomatic fruit there are significant decreases in soluble solids including fructose, glucose, sorbitol, and, in some cultivars, citric acid concentration (Wilks and Milbrath 1956; Wright et al. 2021b) as well as oil content in seeds (Wilks and Milbrath 1956).

Foliar symptoms on sweet and sour cherry are phytoplasma strain, host cultivar, and environment specific, with chlorosis, curling, reduction in leaf size, and premature leaf drop reported during the growing season, and bronzing or anthocyanosis along the midrib and basal veins late in the season followed by leaf drop (Rawlins and Horne 1931; Rawlins and Thomas 1941; Wilks and Milbrath 1956), while in other cases leaf size, color and shape will remain normal throughout the season (Harper, *unpublished data*). In some cases, enlarged stipules may be observed on leaves of infected trees (Fig. 1B) (Thomson et al. 1993).

In the late stages of the infection cycle, heavily infected cherry trees begin to exhibit bare limbs and dieback in spring, with uneven bud development and proliferation of small, pale leaves, which also can be strain specific (Purcell et al. 1987; Rawlins and Thomas 1941; Wright et al. 2021b). One unique reaction of '*Ca*. P. pruni' infecting

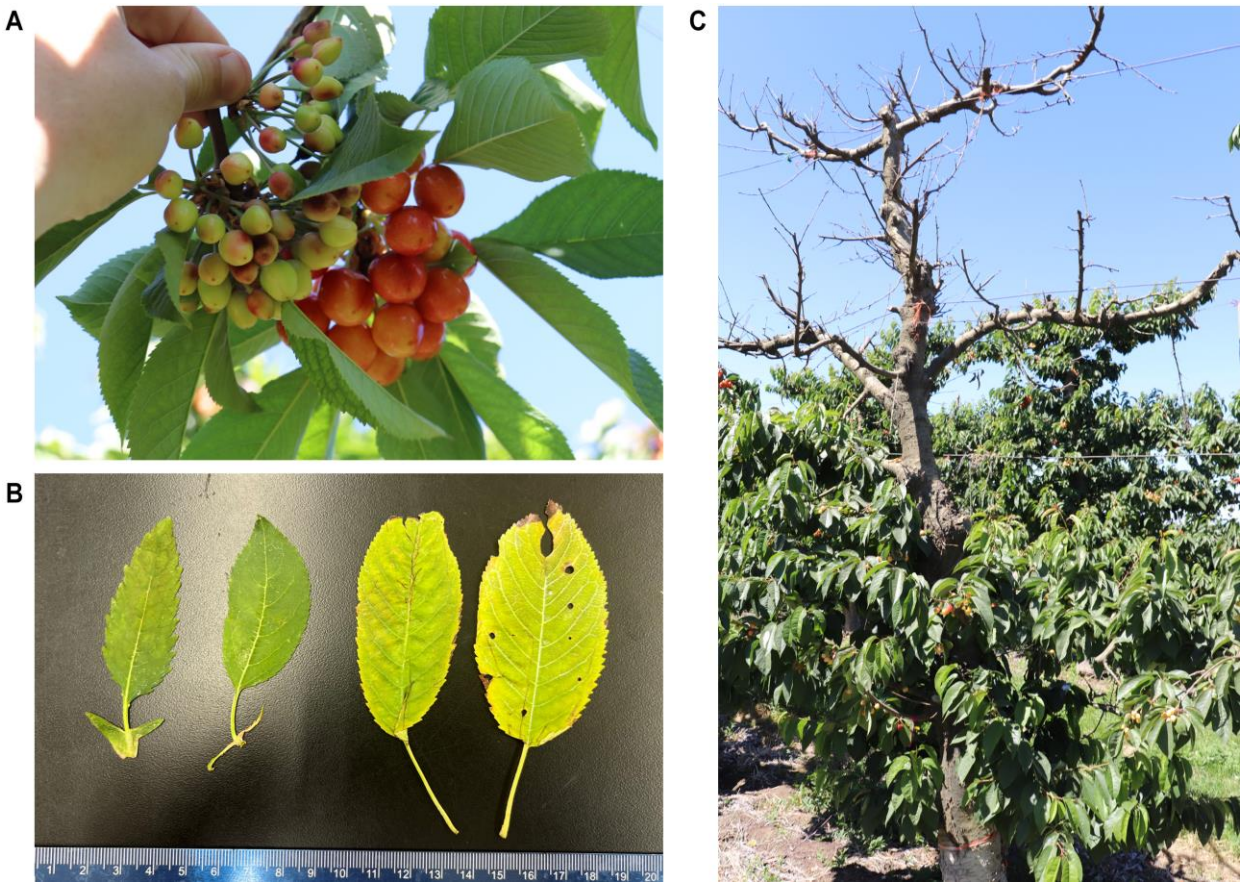

FIGURE 1

X-disease symptoms induced by severe '*Candidatus* Phytoplasma pruni' infection in sweet cherry (*Prunus avium*). **A,** Reduced fruit size, color, and shape relative to normally developed fruit; **B,** enlarged leaf stipules; and **C,** decline and dieback of limbs.

sweet or sour cherry is the decline and death of infected trees on *P. mahaleb* rootstock cultivars. This is a hypersensitive response that induces the breakdown of phloem in or around the graft union (Rawlins and Parker 1933; Rawlins and Thomas 1941; Uyemoto 1989) leading to collapse and death of the scion. Decline is preceded by foliar chlorosis and premature leaf drop, although it should be noted that the rate of decline is dependent on plant age and the infecting strain and can range from months to several years after infection (Rawlins and Parker 1933; Richards and Cochran 1956; Wilks and Milbrath 1956). Decline of sweet or sour cherry on *P. mahaleb* rootstock is not a universal reaction, with certain strains not inducing decline symptoms (Jensen 1956), therefore *P. mahaleb* should not be regarded as a reliable indicator or disease management solution.

Symptoms on peaches and nectarines. Symptoms of what was originally termed "X-disease" or the "yellow-red virosis of peach" in Connecticut and New York (Palmiter and Hildebrand 1943; Stoddard 1938), "Western-X decline" in Utah (Richards and Cochran 1956), and "leaf-casting" in California (Thomas et al. 1940) were reported in the late 1920s and early 1930s. In peach and nectarine, symptoms occur on both the leaves and fruit, and, as the infection progresses, trees may exhibit decline and eventually die (Palmiter and Hildebrand 1943; Richards and Cochran 1956; Wright et al. 2022a). As with cherry, symptoms first appear on one limb, or part of a limb, progressing systemically over the next year or years until the entire tree shows foliar, fruit, or decline symptoms (Marcone et al. 2014), although multiple authors have noted that there may be a "sidedness" to symptoms, with one side or leader heavily symptomatic or even dead, while the other side remains mild to asymptomatic (Richards and Cochran 1956; Wright et al. 2022a). Uneven distribution of phytoplasma within the tree is the likely cause of this phenomenon as symptom severity has been correlated with '*Ca*. P. pruni' titer in or near the affected tissues (Wright et al. 2022a).

Foliar symptoms appear in late spring to early summer on infected trees, beginning as chlorotic blotches on the leaves near the midrib or margins (Richards and Cochran 1956), gradually spreading to encompass the entire leaf, with epinasty and the leaf margins curling inwards to form a reticulate shape (Fig. 2A) (Palmiter and Hildebrand 1943; Richards and Cochran 1956; Wright et al. 2022a); chlorosis correlates with reduced foliar chlorophyll-a and -b as well as carotenoid content (Wright et al. 2022a). Water-soaked areas appear on the leaf blade in summer and late autumn, which become necrotic, starting with chlorosis and shifting to brown or reddish in appearance, and leading to what has been described as a shot-hole or leaf-casting where the tissue dies and irregularly shaped holes are presented in the blade or near the margins (Fig. 2B) (Palmiter and Hildebrand 1943; Richards and Cochran 1956; Thomas et al. 1940); the appearance of these symptoms correlates with the expression of leaf senescence pathways (Wright et al. 2022a). Premature leaf drop often occurs on '*Ca*. P. pruni' affected limbs, followed by fruit drop (Palmiter and Hildebrand 1943).

Fruit symptoms on peach and nectarine are characterized as delayed maturation and reduced fruit size relative to normal, uninfected fruit at harvest (Fig. 2C) (Wright et al. 2022a). Fruit may be distorted and have a bitter flavor that may be corelated, as with cherry, with lower sugar content (Palmiter and Hildebrand 1943; Wright et al. 2022a). External fruit symptoms include red streaking of the fruit and suture, the latter characteristic of the "red suture" disease in the southeastern United States (Scott and Zimmerman 2000), and distortions of the fruit skin, taking on a lumpy or ropey appearance (Wright et al. 2022a). Fruit often remains underdeveloped and unmarketable at harvest, and seeds in such fruit are nonviable (Richards and Cochran 1956).

FIGURE 2

Symptoms of the X-disease phytoplasma on peach (*Prunus persica*). **A,** Early-season foliar chlorosis, curling, and epinasty (from Wright et al. 2022a); **B,** late-season foliar chlorosis and shot-holing; **C,** distorted and smaller, immature fruit at harvest (from Wright et al. 2022a); **D,** disease affecting one limb or scaffold; and **E,** poor growth and dieback of trees in later stages of infection.

Decline and death of infected limbs occurs over the winter, with either poor growth and rosetting in spring, or no growth at all, followed by the appearance of dead wood (Palmiter and Hildebrand 1943). Premature bloom has also been observed in heavily infected trees, although this will rarely result in significant fruit set (Wright et al. 2022a) or the fruit will mummify on the tree (Palmiter and Hildebrand 1943). As described above, decline and death symptoms often display a "sidedness," with one limb, leader, or side dying before the rest (Fig. 2D and E) (Richards and Cochran 1956; Wright et al. 2022a). Decline is irreversible, and dead limbs do not recover. How rapidly a tree declines and dies may depend on the peach cultivar infected, the infecting strain of '*Ca*. P. pruni', and environmental conditions; trees may die as rapidly as 2 to 3 years after infection or may take as long as long as 20 years to die (Marcone et al. 2014; Palmiter and Hildebrand 1943; Richards and Cochran 1956; Wright et al. 2022a).

Symptoms on chokecherry. Outside of commercially grown species, one of the best described and earliest identified *Prunus* hosts is chokecherry (*Prunus virginiana*). In this species, infection is presented as delayed foliation and leaf rolling in early summer, followed by chlorosis that spreads from the margins and leaf tip towards the base and may become anthocyanotic as the season progresses. Terminal rosetting or witches' broom symptoms may be observed in the second year after infection, with decline and death occurring as early as 4 years after infection (Gilmer et al. 1954; Palmiter and Hildebrand 1943). Unlike X-disease phytoplasma in peach, shot holing and premature leaf drop does not occur in chokecherry (Palmiter and Hildebrand 1943). In Utah, Richards and Cochran (1956) reported that fruit set was reduced, primarily in plants at higher elevations, and fruit appeared small, pointed, and pink to pale red rather than the dark red of normal fruit, and that seeds failed to form in the affected fruit.

Reddening symptoms have been described on both Eastern (*P. virginiana* var. *virginiana*) and Western (*P. virginiana* var. *demissa*) chokecherry (Gilmer et al. 1954; Palmiter and Hildebrand 1943; Wilks and Milbrath 1956), although work by Gilmer et al. (1954) suggested that symptom type and severity may be influenced by the infecting strain of '*Ca*. P. pruni'. They defined three strains based on symptoms expressed: The first produced severe anthocyanosis, rosetting, and witches' broom of terminal and auxiliary buds; reduced fruit load that failed to mature; and decline and tree death at 4 years post inoculation. The second was less severe, with slight anthocyanosis leading the leaves to appear bronze-yellow, while fruit and rosetting symptoms remained similar. Unlike the first strain, however, decline and tree death were delayed. Finally, the third type produced anthocyanosis although its onset was slower, rosetting was not observed, and decline was slight (Gilmer et al. 1954). It should also be noted that in the current Pacific Northwest outbreak, infections of *P. virginiana* are of the latter type: primarily asymptomatic, with no rosetting, and minor chlorosis or anthocyanosis observed late in the season (Harper et al., *unpublished data*).

Symptoms on other *Prunus* species. In contrast to the well characterized symptoms produced on cherry, peach, and chokecherry, the symptoms expressed on other *Prunus* species are less pronounced and less characteristic of X-disease infection than a reaction to any number of pathogens. For example, infected plum (*P. domestica*) expresses weak foliar chlorosis, with bronzing and rosetting in select cultivar and '*Ca*. P. pruni' strain combinations (Simonds 1949). In long-term infections, plum fruit maturation may be delayed, resulting in smaller fruit (Harper, *unpublished data*).

Chokecherry-like foliar anthocyanosis and rosetting, followed by decline or tree death, has been reported on sand cherries (*P. besseyi* and *P. pumilia*), bush cherries (*P. japonica* and *P. glandulosa*), Amur chokecherry (*P. maackii*), Bird cherry (*P. padus*), Nanking cherry (*P. tormentosa*) and Beach plum (*P. maritima*) (Gilmer et al. 1954). Foliar chlorosis, with or without terminal growth stunting, has been observed on apricot (*P. armeniaca*), almond (*P. amygdalus*), and wild goose plum (*P. munsoniana*) (Gilmer

et al. 1954; Stoddard 1947), while rosetting and stunting alone was observed on Japanese cherries (*P. serrulata* and *P. sieboldi*) (Gilmer et al. 1954).

Several *Prunus* species can be infected by '*Ca*. P. pruni' but have not been reported to produce symptoms, or the symptoms are uncharacterized, including American plum (*P. americana*), Mahaleb cherry (*P. mahaleb*), Newport plum (*P. cerasifera*; syn. *P. newporti*), pin cherry (*P. pensylvanica*), blackthorn (*P. spinosa*), and hollyleaf cherry (*P. ilicifolia*) (Gilmer and Blodgett 1976; Gilmer et al. 1954). Interspecific hybrids, including common rootstock species such as Krymsk 5 or 6 (*P. cerasus* × (*P. cerasus* × *P. maackii*), and Gisela 6 or 12 (*P. cerasus* × *P. canescens*), can be infected by '*Ca*. P. pruni', but their symptoms have not been as well characterized (Uyemoto et al. 1991; Wright et al. 2021b).

Symptoms on non-*Prunus* species. The X-disease phytoplasma can also produce symptoms on a range of crop and noncrop plant species outside of the genus *Prunus*. Many of these were identified through experimental inoculation, whereas others were identified in the wild. Foliar chlorosis of variable severity was found to be the most common symptom in experimentally inoculated species ranging from celery (*Apium graveolens*) to periwinkle (*Vinca rosea*) and English plantain (*Plantago lanceolata*) (Chiykowski and Sinha 1982; Jensen 1971b). Witches' broom was noted on cinquefoil (*Potentilla intermedia*), while other species exhibited reduction in leaf size, distortion of leaf margins, and, in some cases, decline and death (for a full overview see Jensen [1971b] and Chiykowski and Sinha [1982]).

One of the few discoveries of a symptomatic, naturally infected, non-*Prunus* species was milkweed (*Asclepias styrica*) in New York, presenting small chlorotic leaves on rosetted shoots (Gilmer 1960). Subsequent discoveries of '*Ca*. P. pruni' in naturally infected non-*Prunus* species have been asymptomatic, or symptoms have not been characterized (Molnar et al. 2022). The latter is particularly evident with annual and biennial broadleaf plants in the orchard environment where they are subject to multiple biotic and abiotic stresses. Recent exceptions include the finding of '*Ca*. P. pruni' 16SrIII-A positive poinsettia (*Euphorbia pulcherrima*) expressing reduced bract size and leaf edge distortion in commercial flower production in Ontario (Arocha Rosete et al. 2021), and more significantly, in apple (*Malus domestica*), where it induced smaller fruit, foliar proliferation, and anthocyanosis near the midvein late in the growing season (Nikolaeva et al. 2017).

III. Host Range and Transmission

The epidemiology of the X-disease phytoplasma in both the orchard and extra-orchard environment is primarily defined by two factors: (i) the *Prunus* and non-*Prunus* host species of the phytoplasma, and (ii) how the phytoplasma is transmitted between individual plants, either by the grafting of infected tissue to an uninfected or healthy tree, or, more importantly, by invertebrate vectors (Fig. 3).

***Prunus* host species.** The most economically significant crop and wild perennial hosts of '*Ca*. P. pruni' isolates in North America are members of the genus *Prunus* (Rosaceae: Amygdaleae). From both experimental inoculation and surveys of free-living or commercially grown plants presumably inoculated by leafhopper vector species, the majority of *Prunus* spp. examined to date are systemically infectable by the phytoplasma irrespective of whether they are native to North America or are introduced from Europe or Asia (Table 2) (Gilmer et al. 1954; Rawlins and Horne 1931; Rawlins and Parker 1933; Simonds 1949; Stoddard 1947; Uyemoto et al. 1991).

There are four major subgenera within the genus *Prunus* (Hodel et al. 2021) excluding hybrids, and susceptible species have been found in all (Table 2), suggesting a broad susceptibility to this pathogen, although symptom severity varies considerably. There is some disagreement between studies as to whether individual species, or cultivars thereof, are resistant, tolerant, or immune (Gilmer et al. 1954; Rawlins and

Thomas 1941; Uyemoto et al. 1991), which may be the result of differences in the X-disease phytoplasma strain used, inoculum titer, environmental conditions, and the length of the observation period. For example, a number of *P. avium* cultivars, such as Angela, Black Republican, and Utah Giant, have been reported as resistant (Thompson and Wadley 1981), but more recent surveys (Harper et al., *unpublished data*), have found at least the latter two to be susceptible. The only species tested that are reported to not be infectable are *P. serotina* (black cherry) (Gilmer et al. 1954; Uyemoto et al. 1991) and *P. emarginata* (bitter cherry) (Rawlins and Thomas 1941), although given that other members of subgenera *Padus* and *Cerasus*, respectively, are susceptible, further study is needed before considering these species immune.

Prunus host species are cultivated for agricultural production and as ornamental or landscape plants in the orchard and extra-orchard environments, respectively, therefore potential reservoirs for '*Ca*. P. pruni' infection are present nationwide. Ornamentals may be the most cryptic and potentially difficult to manage reservoir source because X-disease symptoms may be either easily confused for other biotic or abiotic factors or may be assumed to be the normal growth pattern for the plant; for example, Yoshino cherry growing in the Yakima botanical gardens in 2022 were heavily infected but no symptoms were noted (Molnar, *unpublished data*).

Free-living native *Prunus* spp., such as chokecherry (*P. virginiana* subsp. *virginiana* and subsp. *demissa*) and American plum (*P. americana*) have been long recognized as important sources of infection (Gilmer and Blodgett 1976; Guo et al. 1996; Palmiter and Hildebrand 1943; Richards and Cochran 1956). The former is distributed across North America, *P. virginiana* subsp. *virginiana* in the eastern and midwestern states and *P. virginiana* subsp. *demissa* in the western states (Fig. 4), while *P. americana* is found in the eastern states, Appalachia, and into the Midwest (Fig. 4); other wild *Prunus* sp. are more limited in distribution (Volk 2019).

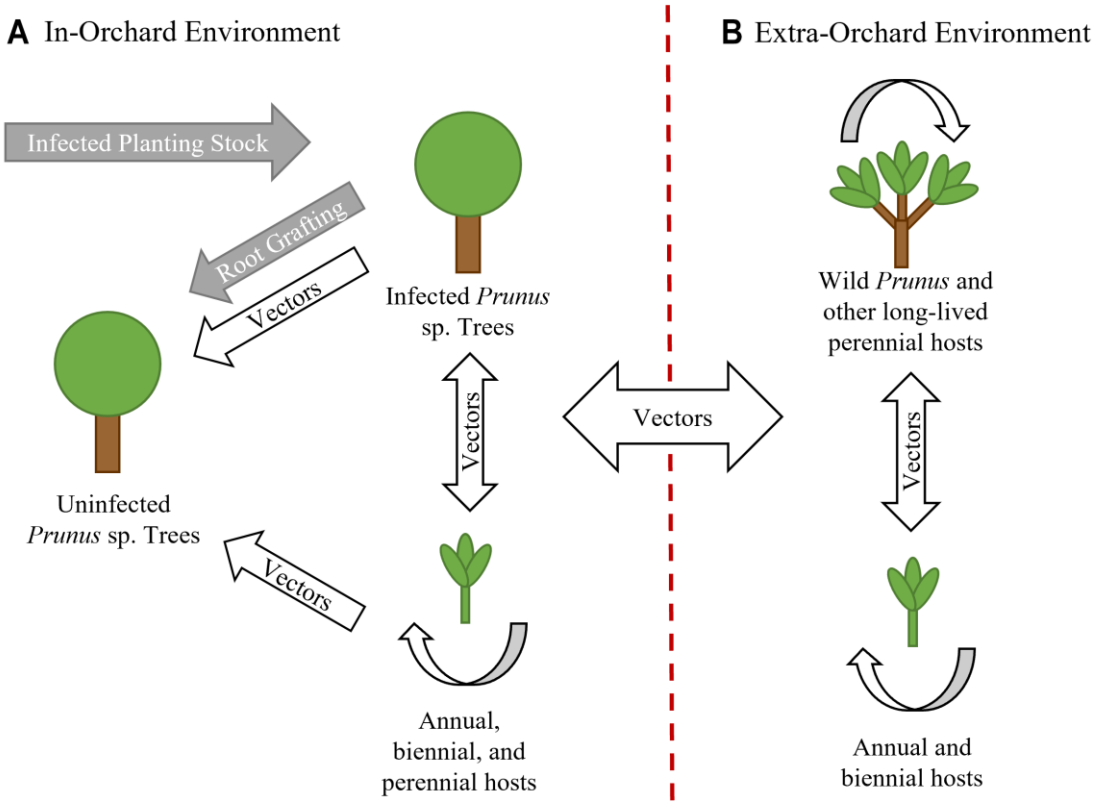

FIGURE 3
Diagram of potential X-disease phytoplasma epidemiology in the **A,** orchard and **B,** extra-orchard environment. White arrows represent vector transmission of the phytoplasma and gray grafting or propagative transmission.

Non-*Prunus* host species. Isolates of the X-disease subgroup (16SrIII-A) of '*Ca*. P. pruni' also have a wide host range outside of the genus *Prunus* that encompasses economically important crops, backyard and garden plants, and free-living or wild annuals, biennials, and perennials. This phytoplasma has been reported, through either experimental inoculation or detection of natural in-field infections, to infect a total of 45 different species from 19 families (Table 3) (Arocha Rosete et al. 2021; Chiykowski

TABLE 2
Host species of '*Candidatus* Phytoplasma pruni' within the genus *Prunus* as determined by either experimental inoculation or detection of natural infection

Subgenus	Species	Common name	First identified by[a]	Symptoms described	Reference
Amygdalus	*P. amygdalus*	Almond	Exp. inoc.	Yes	Stoddard (1946)
	P. mira	Tibetan peach	Exp. inoc.	Yes	Rawlins and Thomas (1941)
	P. mira × *P. persica*	N/A	Exp. inoc.	Yes	Rawlins and Thomas (1941)
	P. persica	Peach/nectarine	Nat. infect.	Yes	Palmiter and Hildebrand (1943)
Cerasus	*P. cerasus*	Sour cherry	Nat. infect.	Yes	Rawlins and Parker (1933)
	P. fructicosa	Dwarf cherry	Exp. inoc.	Yes	Uyemoto et al. (1991)
	P. lannesiana	Oshima cherry	Exp. inoc.	Yes	Gilmer et al. (1954)
	P. mackii	Amur chokecherry	Exp. inoc.	Yes	Gilmer et al. (1954)
	P. mahaleb	Mahaleb	Nat. infect.	No	Rawlins and Parker (1933)
	P. pensylvanica	Pin cherry	Exp. inoc.	Yes	Gilmer et al. (1954)
	P. serrulata	Japanese cherry	Exp. inoc.	Yes	Gilmer et al. (1954)
	P. avium	Sweet cherry	Nat. infect.	Yes	Rawlins and Horne (1931)
	P. × *schmittii*	Schmitt's cherry	Exp. inoc.	No	Uyemoto et al. (1991)
	P. × *yeodensis*	Yoshino cherry	Nat. infect.	No	Shires et al., *unpublished data*
Padus	*P. ilicifolia*	Hollyleaf cherry	Nat. infect.	No	Gilmer and Blodgett (1973)
	P. padus	Bird cherry	Exp. inoc.	Yes	Gilmer et al. (1954)
	P. virginiana	Chokecherry	Nat. infect.	Yes	Palmiter and Hildebrand (1943)
Prunus	*P. americana*	American plum	Exp. inoc.	No	Stoddard (1946)
	P. armeniaca	Apricot	Exp. inoc.*	Yes	Simmonds (1949)
	P. besseyi	Sand cherry	Exp. inoc.	Yes	Stoddard (1946)
	P. cerasifera	Newport plum	Exp. inoc.	Yes	Gilmer et al. (1954)
	P. communis × *P. fenzliana*	N/A	Exp. inoc.	Yes	Rawlins and Thomas (1941)
	P. davidiana × *P. communis*	N/A	Exp. inoc.	Yes	Rawlins and Thomas (1941)
	P. domestica	European plum	Exp. inoc.*	Yes	Simmonds (1949)
	P. glandulosa	Chinese bush cherry	Exp. inoc.	Yes	Gilmer et al. (1954)
	P. japonica	Japanese bush cherry	Exp. inoc.	Yes	Stoddard (1946)
	P. maritima	Beach plum	Exp. inoc.	Yes	Gilmer et al. (1954)
	P. pumila	Sand cherry	Exp. inoc.	Yes	Gilmer et al. (1954)
	P. ruvilaris	Wild goose plum	Exp. inoc.	Yes	Stoddard (1946)
	P. salicinia	Asian plum	Exp. inoc.*	Yes	Simmonds (1949)
	P. spinosa	Blackthorn	Exp. inoc.	No	Gilmer et al. (1954)
	P. tormentosa	Nanking cherry	Exp. inoc.	Yes	Gilmer et al. (1954)
	Prunus × *dunbari*	Dunbar plum	Exp. inoc.	Yes	Gilmer et al. (1954)
Hybrid	*P. avium* × *P. canesens*	Gisela 196/4	Exp. inoc.	Yes	Uyemoto et al. (1991)
	P. avium × *P. pseudocerasus*	Colt	Exp. inoc.	No	Uyemoto et al. (1991)
	P. canescens × *P. cerasus*	N/A	Exp. inoc.	Yes	Uyemoto et al. (1991)
	P. canescens × *P. fructicosa*	G448	Exp. inoc.	Yes	Uyemoto et al. (1991)
	P. cerasus × *P. fructicosa*	N/A	Exp. inoc.	No	Uyemoto et al. (1991)
	P. × *dawykensis*	Damil	Exp. inoc.	Yes	Uyemoto et al. (1991)

[a] An asterisk indicates subsequent detection of natural infection in an experimentally inoculated species. Exp. inoc. = experimental inoculation, and Nat. infect. = natural infection.

and Sinha 1982; Gilmer 1960; Jensen 1971b; Shires et al. 2022; Weathers and Cochran 1950). Species from the families Asteraceae and Brassicaceae are most heavily represented (Table 3).

Many of these species are found in the orchard or extra-orchard environment where commercial *Prunus* spp. are grown and could act as reservoirs for further spread; it has been repeatedly reported that many of the common leafhopper vector species of '*Ca*. P. pruni' in North America feed readily on common annual and biennial species such as clover, alfalfa, dandelion, and other weeds (Cooper et al. 2022; Nielsen 1957; Van Steenwyk et al. 1995), and their absence can reduce leafhopper accumulation in orchard environments (McClure 1980a; Purcell and Elkinton 1980).

Of particular epidemiological significance are species that could carry the phytoplasma from season to season, including overwintering weeds such as dandelion or mallow, which are near ubiquitous in both orchard and extra-orchard environments nationwide unless heavy control measures are applied. Also important are perennial crops such as apple (*Malus* sp.) (Nikolaeva et al. 2017). The climactic zones of commercial stone and pome fruits overlap, particularly in the northern half of the country, and orchards of both are found in close proximity, which presents a particular management conundrum as unsynchronized insecticide treatments have been observed to drive leafhoppers from crop to crop (Shires et al., *unpublished data*).

In contrast, to date there have been few noncrop perennial trees or shrubs identified as hosts, although this is more likely due to prior outbreaks predating the development of PCR assays for pathogen identification (Lee et al. 1992) than an absence of wild host species. Indeed, in the latest outbreak in the Pacific Northwest, sagebrush (*Artemisia tridentata*) was identified as a host (Molnar et al. 2022), which presents a potential phytoplasma reservoir from Montana to Washington and Nevada, with scattered distribution in Colorado, Utah, and eastern California (Fig. 4).

Graft transmission. Graft transmission involves the deliberate or natural attachment and fusion of tissue, forming a contiguous vascular connection through which phytoplasma can move. This can occur through either root grafting or through propagative practices. The former occurs naturally through roots of neighboring trees encountering one another due to proximity or growth and fusing together due to crushing and fusion of cortical cells (Mudge et al. 2009). As the root graft develops,

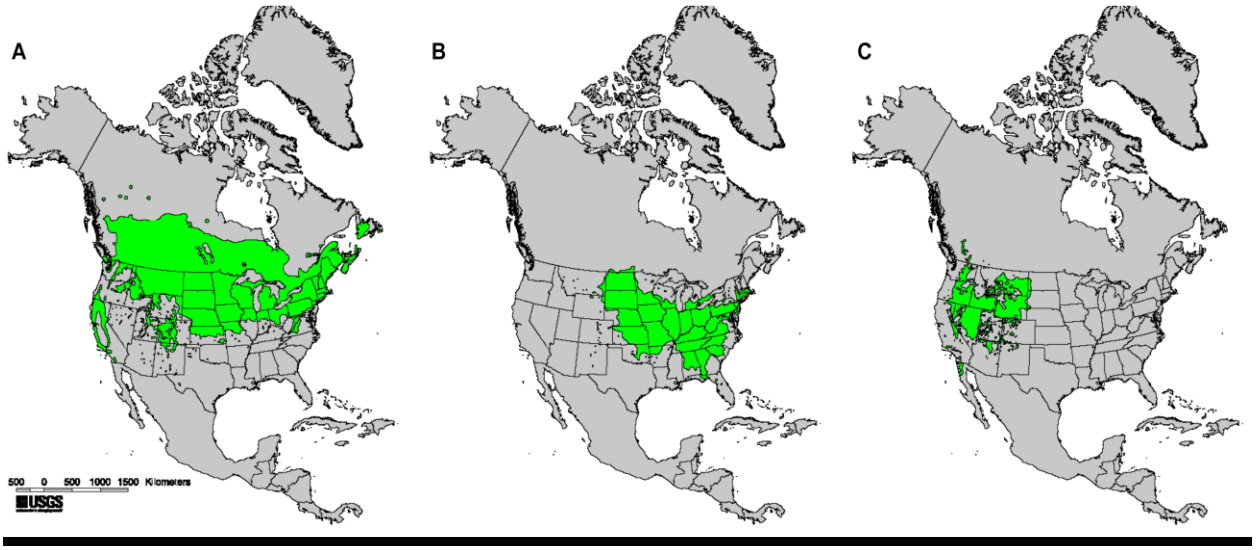

FIGURE 4
Geographic distribution of select noncrop perennial tree hosts of the X-disease in North America. **A,** Chokecherry (*Prunus virginiana*), **B,** American plum (*Prunus americana*), and **C,** big sagebrush (*Artemisia tridentata*). Images from the U.S. Geological Survey (Fryer 2018).

TABLE 3
Non-*Prunus* hosts of '*Candidatus* Phytoplasma pruni' (16SrIII-A) reported in North America by either experimental inoculation or detection of natural infection

Family	Species	Common name	First identified by[a]	Location	Symptoms described	Reference
Amaranthaceae	*Chenopodium album*	White goosefoot	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
	Gomphrena globosa	Globe amaranth	Exp. inoc.	U.S.A.	Yes	Jensen (1971)
Apiaceae	*Apium graveolens*	Celery	Exp. inoc.	U.S.A.	Yes	Jensen (1956)
	Conium maculatum	Poison hemlock	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
	Coriandrum sativum	Coriander	Exp. inoc.	U.S.A.	Yes	Jensen (1971)
	Daucus carota	Carrot	Exp. inoc.	U.S.A.	Yes	Weathers and Cochran (1950); Gilmer (1960)
	Petroselinum crispum	Parsley	Exp. inoc.	U.S.A.	No	Weathers and Cochran (1950); Gilmer (1960)
Apocynaceae	*Asclepias styrica*	Milkweed	Nat. infect.	U.S.A.	Yes	Gilmer (1960)
	Catharanthus roseus	Periwinkle	Exp. inoc.	U.S.A.	Yes	Weathers and Cochran (1950); Gilmer (1960)
Asteraceae	*Ambrosia artemisiifolia*	Ragweed	Exp. inoc.	Canada	No	Chiykowski and Sinha (1982)
	Artemisia tridentata	Big sagebrush	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
	Calendula officinalis	Marigold	Exp. inoc.	Canada	No	Chiykowski and Sinha (1982)
	Callistephus chinensis	China aster	Exp. inoc.	U.S.A.	Yes	Jensen (1971)
	Chrysanthemum carinatum	Chrysanthemum	Exp. inoc.	U.S.A.	Yes	Jensen (1971)
	Matricaria maritima	Barnyard daisy	Exp. inoc.	Canada	No	Chiykowski and Sinha (1982)
	Taraxacum sp.	Dandelion	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
Brassicaceae	*Brassica nigra*	Black mustard	Exp. inoc.	Canada	No	Chiykowski and Sinha (1982)
	Brassica oleracea	Cauliflower	Exp. inoc.	U.S.A.	Yes	Jensen (1971)
	Brassica rapa subsp. *rapa*	Turnip	Exp. inoc.	U.S.A.	Yes	Jensen (1971)
	Brassica rapa subsp. *japonica*	Field mustard	Exp. inoc.	Canada	No	Chiykowski and Sinha (1982)
	Descurainia sophia	Flixweed	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
	Raphanus sativus	Radish	Exp. inoc.	U.S.A.	Yes	Jensen (1971)
	Sisymbrium altissimum	Tumble mustard	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
Caryophyllaceae	*Stellaria media*	Chickweed	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
Euphorbiaceae	*Euphorbia pulcherrima*	Poinsettia	Nat. infect.	Canada	Yes	Arocha Rosete et al. (2021)
Fabaceae	*Lotus corniculatus*	Birdsfoot trefoil	Exp. inoc.	Canada	No	Chiykowski and Sinha (1982)
	Medicago sativa	Alfalfa	Exp. inoc.	U.S.A.	No	Jensen (1971)
	Trifolium pratense	Red clover	Exp. inoc.	Canada	No	Chiykowski and Sinha (1982)
	Trifolium repens	White clover	Exp. inoc.*	Canada	Yes	Chiykowski and Sinha (1982)
Geraniaceae	*Erodium moschatum*	Filaree	Exp. inoc.	U.S.A.	Yes	Jensen (1971)
Malvaceae	*Malva* sp.	Mallow	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
Melanthiaceae	*Trillium* sp.	Trillium	Nat. infect.	Canada	Yes	Arocha Rosete et al. (2016)
Oleaceae	*Syringa* × *josiflexa*	Lilac	Nat. infect.	Canada	No	Green et al. (2015)
Plantaginaceae	*Plantago lanceolata*	English plantain	Exp. inoc.*	Canada	No	Chiykowski and Sinha (1982)
	Plantago major	Broadleaf plantain	Exp. inoc.*	U.S.A.	No	Jensen (1971)
Poaceae	*Hordeum murinum*	Hare barley	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
Polygonaceae	*Polygonum aviculare*	Prostrate knotweed	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
Rosaceae	*Agrimonia eupatoria*	Common agrimony	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
	Malus domestica	Apple	Nat. infect.	U.S.A.	Yes	Nikolaeva et al. (2016)
	Potentilla intermedia	Cinquefoil	Exp. inoc.	Canada	No	Chiykowski and Sinha (1982)
	Rosa sp.	Rose	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*
Salicaceae	*Salix* sp.	Willow	Exp. inoc.	U.S.A.	No	Jensen (1971)
Solanaceae	*Nicotiana tabacum*	Tobacco	Exp. inoc.	U.S.A.	No	Jensen (1971)
	Solanum lycopersicum	Tomato	Exp. inoc.	U.S.A.	No	Weathers and Cochran (1950); Gilmer (1960)
Zygophyllaceae	*Tribulus terrestris*	Puncturevine	Nat. infect.	U.S.A.	No	Shires et al., *unpublished data*

[a] An asterisk indicates subsequent detection of natural infection in an experimentally inoculated species. Exp. inoc. = experimental inoculation, and Nat. infect. = natural infection.

vascular connections are formed from tree to tree, which allows the transmission of the phytoplasma. *Prunus* spp. rootstock cultivars with large or adventitious root systems, such as *P. avium* cultivar Mazzard (Keane and May 1963) are particularly prone to root grafting and potential transmission whereas rootstocks with smaller root systems, such as the *P. avium* × hybrid 'Gisela', 'Krymsk', or 'Corette' series are less likely to do so unless closely planted (Harper, *unpublished observations*).

In contrast, propagation of infected material occurs either at the nursery or in-field or grower level. This may be inadvertent, in that the propagator does not know that the material, be it scion, rootstock, or both, is infected with '*Ca*. P. pruni', or it may be deliberate due to the plants being a trial or experiment. The latter approach is where most of the data concerning '*Ca*. P. pruni' graft transmission in *Prunus* has been generated, beginning with early host range and pathogenicity studies (Gilmer et al. 1954; Rawlins and Parker 1933; Wilks and Milbrath 1956) using either bud or bark chip grafts, as well as grafting of larger branches. Grafting is a highly efficient means of transmission, as it delivers a higher concentration of inoculum than can be achieved with leafhoppers (Harper et al., *unpublished data*), which accounts for the rapidity of disease onset in experimental studies (Wilks and Milbrath 1956), particularly those using younger trees, versus vector-mediated spread (Rosenberger and Jones 1978).

Finally, the X-disease phytoplasma is also transmitted via dodder (*Cuscuta* sp.) and has been used to experimentally transfer the phytoplasma between infected *Prunus* and herbaceous plants (Gilmer 1960; Weathers and Cochran 1950). While dodder is present across much of North America, it is rare in managed commercial *Prunus* orchards, and transmission by this means may be of limited concern in unmanaged or abandoned orchards, or in the wild.

Vector transmission. X-disease is vectored by a small group of leafhoppers in the sub-family Deltocephalinae, with vector efficacy varying by species (Gold and Sylvester 1982; Jensen 1969), and the relative importance of each species varying by region. The first species identified as a vector was *Colladonus geminatus* (informally referred to as the geminate leafhopper) in Washington State (Wolfe et al. 1951a), and *C. geminatus* was considered the primary vector in Washington and Oregon in an X-disease outbreak in the 1940s and 1950s (Nielson 1968). In contrast, in the 1970s and 1980s the key vector for primary infection was thought to be *Colladonus montanus* (mountain leafhopper), which was found primarily foraging on groundcover plants (Purcell and Elkinton 1980), and secondary infection was driven by *Fieberiella florii*, a predominantly arboreal leafhopper species (Purcell et al. 1987). While it is not clear which subspecies of *C. montanus* was most prominent in California at the time (A. Purcell, *personal communication*), early reports suggested that *C. m. reductus* was a more common subspecies in California than *C. m. montanus* (Cieniewicz et al. 2018; Nielsen 1957). Interestingly, Gold and Sylvester (1982) compared X-disease vector efficiency between a colony maintained by Jensen at the University of California Berkeley for many years to a wild-collected biotype that was raised in the same environment and plants for one year and found that the wild-caught biotype had an approximately 25 to 31% longer median latency period than the insectary biotype (Gold and Sylvester 1982; Jensen 1969). The assumption was that the repeated experiments in the insectary had selected for improved vector efficiency, but it remains unknown if it was natural differences in biotypes or an evolved difference from being in an insectary over many generations. Another vector, *Euscelidius variegatus*, was also common in California (Purcell and Elkinton 1980) but was found to have a longer latency period than *C. geminatus* or *C. montanus*, and a lower likelihood of acquiring '*Ca*. P. pruni' than *C. montanus* (Gold and Sylvester 1982; Jensen 1969). Purcell (1985) collected *E. variegatus*, mostly from grasses within orchards, but none were infective, whereas he was readily able to observe transmission from field collected *F. florii* and *C. montanus* at the same time (Purcell 1985). In a similarly timed X-disease outbreak in Connecticut peaches, a study found that the most common vector was *Scaphytopius*

acutus (sharp-nosed leafhopper), with *Colladonus clitellarius* (saddleback leafhopper) and *Paraphlepsius irroratus* (bespeckled leafhopper) as less common vectors (McClure 1980b).

Interestingly, the key vectors of X-disease (*Colladonus* spp., *S. acutus*, and *P. irroratus*) are all relatively closely related in a clade of new world leafhoppers that evolved from a non-grass feeding shared ancestor (Cao et al. 2022). In contrast, *E. variegatus*, which does not appear to vector X-disease as well as the others, is from a distantly related clade of palearctic leafhoppers within the same subfamily, evolving from a grass-feeding ancestor (Cao et al. 2022). Thus, there might be a genetic underpinning driving X-disease vector capacity. At least two other species (*F. florii* and *Osbornellus borealis*) are also known vectors (Jensen 1957) and are in a different clade than *Colladonus* spp., but it is unclear how efficient they are as vectors compared to *Colladonus* species.

While estimates of the latency period occurred prior to the development of molecular tools that would have improved precision, it is estimated that the latency period for *C. montanus* and *C. geminatus* is approximately 1 month or slightly longer (Gold and Sylvester 1982; Jensen 1969). It has been estimated that it takes approximately 2.5 days of exposure for leafhoppers to acquire the phytoplasma (median acquisition access period) (Whitcomb et al. 1966). Growth chamber experiments found that transmission by *C. montanus* is more likely during daytime, rather than nighttime hours, presumably due to differences in leafhopper feeding activity, as the transmission patterns generally tracked patterns of leafhopper excretion (Gold and Sylvester 1982).

Because X-disease phytoplasma has a wide host range and has been collected from a wide range of plants (Chiykowski and Sinha 1982; Jensen 1971b), the key determinants of whether an insect is an X-disease vector are likely related to the part of the plants fed upon by the insect, and the physiological relationship between the phytoplasma and insect. The phytoplasma is a propagative, persistent pathogen that must move from the vector's gut to the hemolymph, and ultimately to the salivary glands when transmission can occur (Koinuma et al. 2020). While it has not been evaluated in X-disease phytoplasma, specific gut-binding proteins have been identified in '*Ca.* P. asteris' that allow it to bind to the intestinal lining of its leafhopper vectors, allowing access to the vector's hemolymph (Suzuki et al. 2006). It is likely that similar proteins allow '*Ca.* P. pruni' to bind to the gut lining of X-disease vectors and reach the hemolymph, and variation in this gut lining may limit X-disease vector capacity. In addition, X-disease phytoplasma negatively impacts the longevity and reproduction of its vectors (Jensen 1971a), apparently prompting an immune response to the phytoplasma (Lee and Jensen 1963). This immune response may impact the ability of phytoplasma to effectively reach the salivary glands, allowing transmission. For example, an immune response to flavescence dorée phytoplasma (16SrV) appears to inhibit field transmission by *E. variegatus* (Galetto et al. 2018). A similar immune response to X-disease phytoplasma may contribute to a longer latency period and lower effective transmission by *E. variegatus* compared to *C. montanus* (Gold and Sylvester 1982; Jensen 1969), that is observed even when the phytoplasma is directly injected into the leafhopper's hemolymph (Gold and Sylvester 1982).

IV. Monitoring, Detection, and Identification

In cherry, peach, and other stone fruit, annual monitoring for the introduction or spread of X-disease in an orchard is an integral component of disease management. This occurs through a combination of disease scouting and confirmation by PCR, as well as vector trapping and identification.

Scouting for disease symptoms. The primary means of monitoring for the presence of '*Ca*. P. pruni' in cherry or peach orchards is through visual scouting for X-disease symptoms. However, as described below, the approach and timing of scouting differs

between cherry and peach, nectarine, and other stone fruit. Furthermore, local and regional environmental differences in environment and crop production practices should be considered.

In cherry, the absence of reliable and characteristic foliar symptoms means that the fruit is the only tissue suitable for visual diagnosis. This is compounded by symptomatic fruit only being clearly distinct at approximately 2 weeks prior to harvest, depending on climatic conditions (Wright et al. 2021b). Trees should be scouted from the lowest limbs, working upwards towards the canopy, as the phytoplasma accumulates in lower limbs first (Wright et al. 2022b), covering all four quarters of the tree, and marking suspect limbs. Wright et al. (2021b) defined parameters for assessing disease severity on the fruit, accounting for fruit size, color, and shape, although in-field training is recommended as fruit may be small or pale due to abiotic causes including water stress, salinity, shade, and over cropping. X-disease symptoms also appear very similar to little cherry disease (Fig. 5), caused by the eponymous little cherry viruses 1 and 2 (Wright et al. 2021b), and it can be extremely difficult for even highly trained and experienced field scouts to correctly identify which pathogen is the causal agent. In addition, small or pointed cherries may be caused by other cherry-infecting viruses, including Prunus necrotic ringspot virus (Uyemoto and Scott 1992) and tomato ringspot virus (Reinhold and Pscheidt 2023). In case of uncertainty, confirmation by PCR is recommended.

In peach, nectarine, or other stone fruit, scouting is aided by the expression of foliar symptoms on infected limbs or scaffolds, as well as delayed fruit maturation and distortion, or decline and dieback. However, fruit distortion and foliar chlorosis in the absence of shot holing may be the result of peach yellow leaf roll disease, caused by '*Ca*. P. pyri' (Marcone et al. 2014). Given that the symptoms in peach and other stone fruit worsen as the season progresses, observations of characteristic symptoms can occur up to and after harvest. Scouting of peaches is also possible in spring, with severely affected limbs producing abnormal bloom, or simply failing to bloom; confirmation by PCR is recommended later in the season.

The entire orchard must be scouted, for while some vector species are prone to clustering at the edges of orchards (McClure 1982), others penetrate deeply and the first evidence of infection in an orchard may be found away from orchard borders (Harper, *unpublished data*); vector movement and accumulation within an orchard are driven by the groundcover composition (McClure 1982). Should symptomatic trees be found, the neighboring one to two rows of trees should be scouted, particularly if grown on a rootstock prone to root-grafting, such as *P. avium* cultivar Mazzard (Keane and May 1963), or if leafhopper incidence is high.

Finally, it has been found that scouting must be performed annually, as the first symptoms of disease are mild and easily missed (Wright et al. 2021b). The in-season

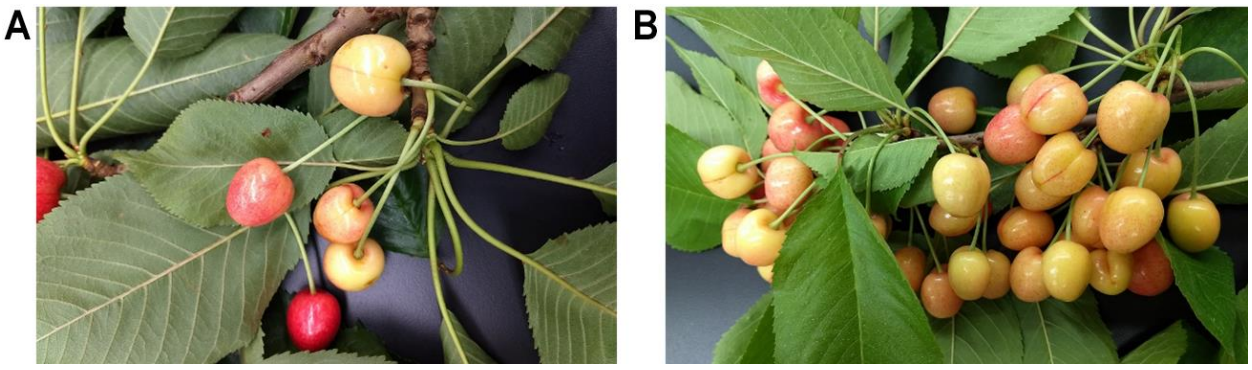

FIGURE 5
Similar severe fruit yellowing and distortion, caused by **A,** little cherry virus 2, and **B,** the X-disease phytoplasma ('*Candidatus* Phytoplasma pruni') on the sweet cherry cultivar Bing at harvest.

timing for scouting should be adjusted to the local environmental conditions since low spring temperatures can suppress early season phytoplasma accumulation, leading to milder or later symptom expression, while warmer temperatures can produce the opposite (Wright et al. 2021b).

Sample collection and diagnostic tools. Sample collection for laboratory diagnosis of '*Ca*. P. pruni' infection in *Prunus* spp. is, as with scouting for disease symptoms, dependent on the stage of the infection (Wright et al. 2021b) and time of year. Based on Wright et al. (2022b), woody stem tissues are viable throughout much of the year, though phytoplasma titer may drop by several orders of magnitude as the tree enters dormancy. The peduncle (fruit stem) is a suitable tissue from the pit hardening to harvest stages of fruit development, with older, fully expanded leaves becoming suitable for detection post-harvest (Wright et al. 2022b). Tissue samples should be collected from symptomatic limbs whenever possible, as the pathogen titer is higher (Wright et al. 2022a, b). When no symptoms are present, or for sampling outside of the fruit development to harvest cycle, woody stem tissue samples should be collected from lower limbs and close to the trunk as phytoplasma accumulation is from the trunk upwards towards sink tissues (Wright et al. 2022b). Roots are also a viable tissue, although they are laborious to collect for routine or large-scale sampling.

Diagnostic assays for '*Ca*. P. pruni' identification are primarily PCR-based, amplifying core conserved genes such as the 16S ribosomal RNA gene or *secA*. Most PCR assays are generic (Bertaccini et al. 2019; Dickinson and Hodgetts 2013; Foissac et al. 2013; Hodgetts et al. 2008; Lee et al. 2010), amplifying multiple phytoplasma species beyond '*Ca*. P. pruni', and, as such, require identification by sequencing of the amplicon and alignment with a reference sequence. The most recent revision of phytoplasma taxonomy (Bertaccini et al. 2022) lists the NCBI GenBank accession numbers for '*Ca*. P. pruni', all of which are derived from a Canadian X-disease phytoplasma isolate from peach, for 16S rRNA, *tufB*, *secA*, *secY*, *rplV-rpsC*, and *groEL* genes, and the percentage nucleotide identities required for assignment to a phytoplasma species. Phylogenetic analysis may be used to separate X-disease or 16SrIII-A isolates from other '*Ca*. P. pruni' subgroups. Complete 16S rRNA gene sequences may also be submitted for identification using the *i*Phyclassifer (Zhao et al. 2009), which can perform both species identification, and through in silico restriction fragment length polymorphism (RFLP) analysis, 16S rRNA subgroup assignment.

Quantitative PCR (qPCR) assays provide a more rapid and sensitive means of detecting the X-disease phytoplasma. This is important as the phytoplasma is at low titer throughout much of the early stages of the infection cycle and unevenly distributed in trees, exacerbated by seasonal fluctuations in titer and distribution which make tissue selection critical (Wright et al. 2022a, b). Generic qPCR assays exist (Christensen et al. 2004; Ito and Suzaki 2017), but as with conventional PCR, require secondary confirmation to determine the phytoplasma species amplified. At time of writing, there is only one species-specific assay available for '*Ca*. P. pruni' detection and identification (Kogej et al. 2020); this has been verified and used extensively in studying and monitoring the recent Pacific Northwest X-disease epidemic.

Finally, both recombinase polymerase amplification (RPA) and loop-mediated isothermal amplification (LAMP) assays for the detection of '*Ca*. P. pruni'/16SrIII phytoplasmas have been published (Aljafer and Dickinson 2021; Villamor and Eastwell 2019). These provide comparable detection sensitivity to nested PCR assays and, given their isothermal chemistries, could be used in the field. However, they are cost prohibitive on a per sample basis, and, as with PCR, subject to the same limitations of uneven in-planta distribution of the phytoplasma during sample collection.

Vector trapping and identification. X-disease vectors can be collected either from sticky traps, sweep nets, or vacuum-powered collection devices such as a Dietrick Vacuum Sampler (DVAC) or other vacuum insect sampling devices. Purcell and Elkington (1980) found that sticky traps collected the most *C. montanus*, followed by

DVACs, and then sweep nets. However, the relative rate of capture with DVACs and sweep nets increased with greater collection effort. Furthermore, the optimal placement of traps likely depends on management of the leafhoppers and the impacts on their feeding location. Nielson (1968) used yellow sticky traps on 5-m tall poles to compare *C. geminatus* flight heights from 2- to 4.5-m high and found highest capture at the lowest heights. Purcell and Elkinton (1980) went further and measured capture of *C. montanus* at 25-cm intervals from 25 cm to 1.25 m and also found the highest counts at the lowest heights (25 and 50 cm from the ground), although leafhoppers were captured at all heights. The authors suggested this high capture at low heights was representative of them feeding commonly on plants in orchard groundcovers but noted that capture at 1.25 m suggested they readily move into trees as well. They found similar results with long mesh sticky traps, although leafhopper capture was much lower (Purcell and Elkinton 1980). In contrast, Van Steenwyk et al. (1990) compared *C. montanus* and *F. florii* capture in traps set at 1.8 and 4.9 m high over the course of a season in an untreated orchard (*C. montanus*) and ornamental firethorn hedge (*F. florii*) and found that while *C. montanus* counts were typically higher at 1.8 m mid-season, counts were higher at 4.9 m in early spring and late fall. The authors interpreted this finding as migration between orchards and overwintering habitats. The counts of *F. florii* were much lower than *C. montanus*, and were typically caught more in higher traps, particularly at the end of the season (Van Steenwyk et al. 1990). It is unclear what caused the differences between counts at high heights observed by Van Steenwyk et al. (1990) compared to highest counts at low trap heights by Nielson (1968) and Purcell and Elkinton (1980), but Van Steenwyk et al. conducted the experiment at a single orchard, which may have impacted the results. In the Midwest United States, using light traps, it was found that another vector, *P. irroratus*, moves into trees at night and then moves down into the groundcover during daylight hours (Larsen and Whalon 1987), although it is unclear whether this movement into trees is to improve communication between leafhoppers, to avoid nocturnal, ground-foraging predators, or something else. A mark-release-recapture experiment in California found that leafhopper capture on yellow sticky traps was highest when leafhoppers were moving north or east, suggesting that leafhoppers were more likely to land on traps when the sun was at their back and reflectance off the trap was greatest (Purcell and Suslow 1982), thus some inclusion of a light source may further improve capture by yellow sticky traps.

Published identification keys distinguishing subfamilies and tribes are available (Dietrich 2005), although recent research suggests some tribes are paraphyletic (Cao et al. 2022), including the *Athysanini*, which contains the X-disease vectors *Colladonus* spp. and *E. variegatus*. An interactive key is also available (http://dmitriev.speciesfile.org/key.asp?key=Cicnymph&i=1&lng=En). The primary vector in California and Washington State, *C. montanus*, is easily identifiable from a yellow transverse stripe across the dorsal of the leafhoppers (Fig. 6), although the general coloration of the insects can vary by season, with darker leafhoppers produced in shorter daylengths (Marsh 1965). This species includes three subspecies, *C. m. montanus*, *C. m. mulsus*, and *C. m. reductus*, with a yellow spot distinguishing *C. m. montanus* from *C. m. reductus*, whereas *C. m. mulsus* can be distinguished from *C. m. montanus* by its longer bifurcate processes and more robust stylar shaft than *C. m. montanus* (Nielsen 1957). Nielson (1957) noted that *C. m. reductus* was the most common subspecies in California, whereas *C. m. montanus* was more common further north, in Washington and British Columbia, but in recent years *C. m. reductus* has been the most common *Colladonus* species or subspecies (Northfield, *unpublished data*).

V. Response

The long history of X-disease affecting U.S. stone fruit production has shaped the response to outbreaks of this pathogen across the country. As the pathogen is endemic

and not subject to monitoring or management at the state or regional level, USDA-APHIS has no active involvement when the disease reemerges in a growing region. Instead, from the outbreaks in 1930s across the country, in the 1970s in California and the Midwest, and since 2010 in the Pacific Northwest, the response at the local level has been led by state and university cooperative-extension agents, state university and federal researchers, and industry groups (Palmiter and Hildebrand 1943; Purcell et al. 1987; Richards and Cochran 1956).

Key to the response has been the formation of formal and informal collaborative networks, such as the Little Cherry and X-disease Task Force comprised of Oregon and Washington state researchers, industry members, and state regulatory representatives in response to the Pacific Northwest outbreak. Researchers and extension agents investigated grower reports of abnormal cherry fruit, as well as foliar chlorosis and dieback in peach, confirming the identity of the causal agents, characterizing the appearance and expression of the disease (Wright et al. 2021a, b, 2022b), and communicating findings back to growers.

Communication and education have been critical to helping growers make informed management decisions. Between 2018 and 2022, researchers and extension specialists held eight online and in-person training sessions and gave 26 presentations to a cumulative 3,986 participants on topics ranging from disease and vector identification to tree removal and chemical control. Digital communication was also important to push information out to growers and pull them to dynamically updated resources. For example, 31 newsletter articles have been distributed to 2,048 listserv subscribers. Ten

FIGURE 6
Examples of leafhopper species that are vectors of the X-disease phytoplasma in North America: **A,** *Colladonus geminatus*, **B,** *Colladonus montanus reductus*, **C,** *Paraphlepsius irroratus*, and **D,** *Scaphytopius acutus*. Images courtesy of C. Reyes-Corral, Washington State University.

webpages provide a constantly available resource for key information. For example, Washington State University's X-disease phytoplasma page (see the web resources section of this document) has received 6,425 unique pageviews at the time of writing. Other digital media, including nine videos and a scouting guide phone application, have helped reach users who increasingly access information via hand-held devices. Traditional media and one-on-one consultations and farm visits were also employed to reach small and less tech-savvy orchardists.

Continuous outreach has been important because in Washington state the disease did not affect all growing regions simultaneously, beginning first in the Yakima Valley then moving northwards (Fig. 7). In Oregon, X-disease has been identified in orchards throughout Wasco and Umatilla Counties, but other cherry-growing regions in Hood River County and the Willamette Valley remain unaffected (Thompson et al., *unpublished data*). Even within each affected growing region, not all growers were affected to the same degree, therefore providing timely evidence-based data on disease spread was essential as a call to action for the growers, and from there to state and federal agencies. Such measures have largely been effective, with 89% of growers surveyed in 2020 stating that they had changed their management practices in response to outreach efforts (DuPont et al., *unpublished data*). Evidence of this is clearly illustrated by the numbers of trees removed by growers, from 10,274 in 2018 to 105,468 in 2020 (Molnar et al. 2022).

VI. USDA Pathogens Permits and Regulatory Issues

As an endemic and suspected native pathogen to the United States, and, in all likelihood, North America, '*Ca*. P. pruni' is not regulated at the federal level and is not included in the phytoplasma species on the priority pest list for the USDA-managed Cooperative Agricultural Pest Survey. However, a PPQ 526 permit is required for the interstate movement of known '*Ca*. P. pruni'-positive material.

At the state level, the X-disease phytoplasma or '*Ca*. P. pruni' are not specifically named in any U.S. state-level exterior quarantine. However, both Oregon and Washington do regulate and exclude *Prunus* species, including *P. americana*, *P. cerasifera*, *P. domestica*, *P. hortulana*, *P. munsoniana*, and *P. salicina* from the majority of eastern and southern U.S. states due to the presence of peach yellows, little peach,

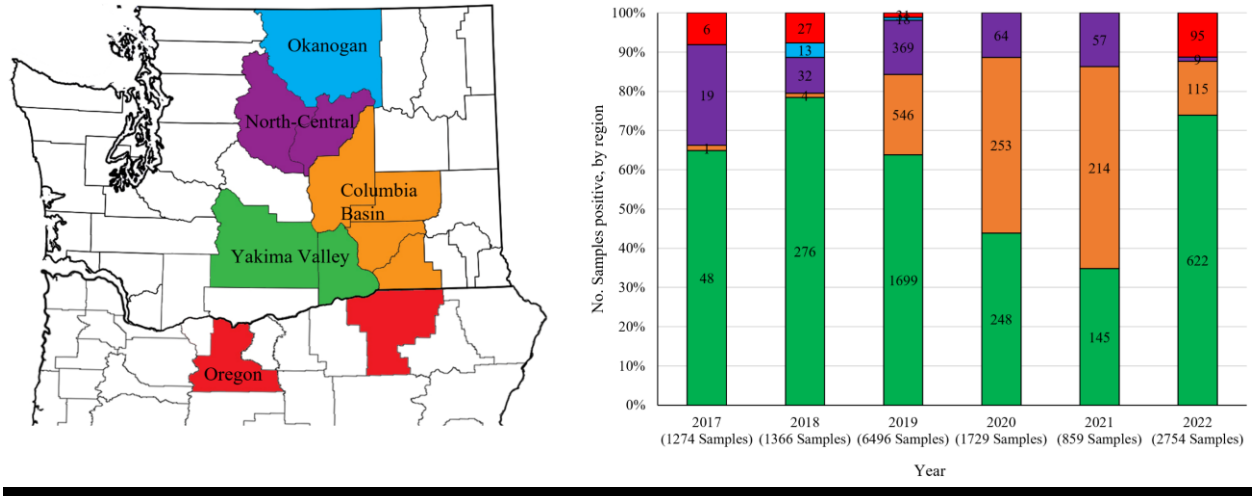

FIGURE 7

X-disease phytoplasma positives detected in major growing regions of Washington (Okanogan, North-Central, Columbia Basin, and Yakima Valley) and Oregon, 2017 to 2022, as determined by PCR. It should be noted that grower practices of scouting versus testing for identification can skew the reported incidence.

and red suture diseases in those regions. However, as the causal agent of the above diseases was identified as '*Ca*. P. pruni' (Scott and Zimmerman 2000), this equates to a de jure, if not de facto, exterior quarantine of X-disease. In California, Oregon, and Washington, '*Ca*. P. pruni' is recognized as being present and is not currently subject to any state-enforced regulatory actions. At time of writing, there are no interior quarantines in effect to prevent the movement of '*Ca*. P. pruni'-infected material within any state.

VII. Economic Impact and Compensation

Economic impacts. There are three major economic impacts of X-disease on stone fruit production: loss of crop yield, loss of trees, and the cost of tree replacement. Yield loss, the fruit that were not harvested due to disease impacts, becomes progressively worse as the pathogen spreads and accumulates in the tree (Wright et al. 2021b). In early stages, most of the tree may be harvested, leaving only the most suspect fruit on the tree, but as diseased fruit on an individual tree reaches approximately 70 to 80%, the average grower tends not to harvest any fruit (G. Bishop, *personal communication*). Tree death or removal magnifies the economic harm as no fruit will be harvested in that year or subsequently. The economic impacts of these two factors have recently been assessed through grower surveys in the Pacific Northwest where between 2015 and 2020 a total of 238,856 cherry and 33,082 peach, nectarine, plum, and apricot trees were removed (Molnar et al. 2022). In terms of yield, this represents 11,408,783 pounds of cherries not harvested, which translates to a $65,047,833 USD loss to growers in the region between 2015 and 2020 (Molnar et al. 2022). Worse, these numbers are likely an underestimate as the survey encompassed only approximately 25% of growers in the region; in a separate survey of orchardists in Wasco County, Oregon, 48% reported removing at least one tree due to X-disease, and 17% reported removing over 100 trees due to infection (Thompson, *unpublished data*).

Replacing lost trees is also a major cost to growers, and establishing and maintaining a cherry orchard until it becomes productive has been estimated at $59,189 to 64,095 per acre (Galinato et al. 2019; Molnar et al. 2022). These costs increase when lost revenue through absence of fruit sales is considered, estimated at $9,000 per acre, therefore growers are faced with a replacement cost of $118,095 per acre across a period of 7 years until the orchard becomes productive (Molnar et al. 2022). These numbers do not account for the likelihood of X-disease phytoplasma infecting the trees before they become productive, which is occurring in the Pacific Northwest, with growers removing entire blocks before recovering the initial cost of investment (Sallato, *unpublished data*).

Compensation. At the time of writing, compensation options for growers and producers are limited. The USDA Farm Service Agency-administered Tree Assistance Program (TAP) provides funding to partially cover (50 to 65%) the cost of replanting trees removed due to X-disease or other natural disasters, although specific criteria must be met in order to access these funds: (i) tree losses of 18% or greater in an orchard must be recorded, and the cause confirmed by diagnostic testing and/or expert assessment; (ii) trees may be herbicide treated or stumped but not removed prior to FSA inspection, and (iii) the trees must be replaced within 1 to 2 years of receiving the funds. Crop loss may also be covered by either private or federal crop insurance, although eligibility and compensation vary from state to state.

VIII. Mitigation and Disease Management

'*Ca*. P. pruni' is well established in the United States and is present in most major stone fruit-growing states across the country, therefore eradication is improbable, if not impossible. Management and mitigation efforts are as follows:

Removal of infected trees. Removal of infected trees was one of the first recognized and most effective control measures for the suppression of past outbreaks of '*Ca*. P. pruni'-associated diseases in stone fruit (Blake et al. 1921; Gilmer and Blodgett 1976; Palmiter and Hildebrand 1943; Purcell et al. 1987; Richards and Cochran 1956; Smith 1894; Van Steenwyk et al. 1995), and indeed, was legally mandated in many East Coast states (Blake et al. 1921; Palmiter and Hildebrand 1943). Infected trees act as sources for further spread through root-grafting to neighboring trees, and as reservoirs for acquisition and transmission of the phytoplasma by leafhopper vector species both within and between orchards. During the California outbreak of the 1970s and 1980s, tree removal was identified as the most significant factor in reducing further spread and was more effective than insecticidal sprays (Van Steenwyk et al. 1995).

Approaches to tree removal have changed little over time (Blake et al. 1921; Palmiter and Hildebrand 1943; Richards and Cochran 1956; Smith 1894; Van Steenwyk et al. 1995), with emphasis on removing the tree during the growing season to ensure that the herbicide used has time to move systemically through the tree and identifying and removing suckers that emerge from the broken root system after tree stumps are taken out. In addition, insecticide applications were required before tree removal as the leafhoppers in the orchard may otherwise be disturbed and disperse.

For growers, tree removal is an economic decision (Molnar et al. 2022), and many choose to retain trees that are mildly symptomatic, or even severely symptomatic, if saleable fruit can be harvested from the tree. In the long term this is detrimental to recovery efforts and may hinder area-wide management as individual growers pursue different levels of tree removal and replacement. Education, tree-removal incentive programs, and, as in the past, legislation may be needed.

Vector control. Because there are no known treatments for X-disease, most efforts have focused on reducing the rate of spread by removing infected trees and reducing vector numbers by applying chemical controls. An evaluation of the effectiveness of an X-disease abatement program in California from 1986 to 1990 found that tree removal protocols explained 71% of the variability in orchard management success (Van Steenwyk et al. 1995). In contrast, variability in chemical control regimes (measured as cumulative insecticide residual time) only explained an additional 6% of the variance in X-disease management success. Similarly, the key to success in overcoming the X-disease outbreak in the Pacific Northwest in the 1950s was thought to be tree removal (Purcell et al. 1987). However, removal of DDT as an option is thought to have led to a resurgence in X-disease incidence in Michigan (Dhanvantari and Kappel 1978).

Currently, there are several commercially available products that work on leafhoppers in the form of insecticidal solutions or particle films. Several active ingredients have shown promise in direct spray, aged residue, and soil drench bioassays. Pyrethrins (MGK PyGanic, 64 fl. oz./acre), thiamethoxam (Syngenta Actara, 2.75 oz./acre), esfenvalerate (Valent Asana XL, 14.5 fl. oz./acre), sulfoxaflor (Corteva Agriscience Transform, 2.75 oz./acre), and imidacloprid (Bayer Crop Science Admire Pro, 2.8 fl. oz./acre) all achieved between 72 and 100% mortality of leafhoppers in multiple assays where insects were directly sprayed with field rate solutions. When insects were exposed to cherry leaves treated at the field rate and allowed to age for 1, 3, 7, and 14 days, mortality was lower overall and did not differ significantly between residue ages. Aged esfenvalerate (Valent Asana XL, 14.5 fl. oz./acre) residues achieved an overall mortality of 58.1% (± 2.2) and aged thiamethoxam (Syngenta Actara, 2.75 oz./acre) residues achieved an overall mortality of 88% (± 1.2). Early soil drench and leaf uptake bioassays suggest that imidacloprid (Bayer Crop Science Admire Pro, 2.8 fl. oz./acre) and thiamethoxam (Syngenta Platinum 75SG, 3.67 oz./acre) could be useful in a leafhopper management program, with 73 to 98% of exposed leafhoppers dying within 24 hours of exposure. Particle films provide a physical barrier to discourage landing and feeding on treated leaves. Two active ingredients, diatomaceous earth (Brandt

Celite 610, 50 lb./acre) and kaolin clay (Arbico Organics Surround WP, 50 lb./acre), effectively repelled leafhoppers from treated surfaces in choice tests. Both ingredients reduced the number of leafhoppers per leaf by more than 80% when compared with untreated leaves (Nottingham and Northfield 2022). In field trials kaolin clay reduced X-disease vector numbers within a plot by 47 to 48% in high pressure blocks and significantly reduced the height at which leafhoppers were caught in traps, suggesting reduced movement into trees (Northfield and Nottingham 2022).

However, a key to application is to adequately time the treatments, accounting for the leafhopper phenology, and the seasonal likelihood of acquisition by leafhoppers from infected plants, and the latency period within leafhoppers. In the Pacific Northwest and Utah, *C. geminatus* and *C. m. reductus* overwinter as eggs (Kaloostian 1956; Marsh 1965; Nielson 1968). In contrast, in California *C. montanus* is reported to overwinter as adults in sugar beets (Purcell et al. 1987). Because the X-disease phytoplasma does not appear to be vertically transmitted (transovarial), leafhoppers must reacquire the phytoplasma in each generation. Similarly, it does not appear to be transmitted via seed, so perennial plants are important sources of vector acquisition each year. In the 1950s in the Pacific Northwest *C. geminatus* exhibited two generations per year, with adults occurring in May and September, respectively. Similarly, in Michigan in the 1970s *P. irroratus* was found to have two generations, with adults of each generation emerging from late June to July and from late September to October, respectively (Taboada et al. 1975). In contrast, in Washington State there are three *C. m. reductus* generations, with the adults emerging in late May or June, August, and late September/October (Northfield, *unpublished data*). X-disease phytoplasma is not readily available for acquisition by leafhoppers feeding on leaves early in the season (Wright et al. 2022b), so X-disease acquisition is greater later in the season, once phytoplasma levels have built up in the leaves (Suslow and Purcell 1982). Indeed, Northfield and Harper (*unpublished data*) have only observed infective *C. geminatus* or *C. m. reductus* leafhoppers in August or later in the year. Interestingly, this late transmission is typically after cherries and in some cases other stone fruit are harvested, meaning the greatest danger of transmission occurs when no fruit are on the trees. Thus, management efforts prior to harvest are deployed to reduce the numbers of leafhoppers building up in the area, and control measures after harvest are to disrupt transmission.

Alternative host management. The X-disease phytoplasma has a wide host range beyond commercially grown stone fruits, and identification and management of reservoir species in the orchard and extra-orchard environment are necessary to reduce the likelihood of pathogen spread. In the orchard environment, perennial, biennial, and annual weeds, including cosmopolitan species such as dandelion (*Taraxacum* sp.), mallow (*Malva* sp.), clover (*Trifolium* sp.), and goosefoot (*Chenopodium album*), are significant hosts for both phytoplasma (Shires et al. 2022) and leafhopper vectors. Removal or suppression of preferred feeding hosts can reduce leafhopper vector incidence and disease spread (Douglas and McClure 1988; McClure et al. 1982) and elimination of broadleaf phytoplasma hosts reduces the likelihood of acquisition of the phytoplasma by apterous leafhopper nymphs (Northfield et al., *unpublished data*). Suppression of weeds may be accomplished by mowing (Douglas and McClure 1988) or by pre-emergent and in-season chemical applications, although Purcell and Elkington (1980) found little to no benefit of mowing or discing groundcover on *C. montanus* numbers. Rather, the authors found even higher numbers of *C. montanus* after mowing, potentially due to the highly nutritious regrowth of weedy hosts. More recently, in Washington field trials, it was found that reducing access to weedy hosts through the use of reusable ground covers reduced leafhopper (primarily *C. m. reductus*) numbers by 81 to 90% (Northfield and Nottingham 2022).

In the extra-orchard environment, many of the same cosmopolitan weed species exist, but are complimented by wild perennial *Prunus* hosts including chokecherry (*P. virginiana*), American plum (*P. americana*), or long-lived non-*Prunus* species such as

sagebrush (*Artemisia tridentata*) and apple (*Malus* sp.) in which the phytoplasma can persist for years. Chokecherry removal was an emphasis of early X-disease mitigation programs (Palmiter and Hildebrand 1943; Richards and Cochran 1956) and in New York, removal of chokecherry in a 500-foot radius of commercial orchards was found to effectively prevent new infections in peach (Palmiter and Hildebrand 1943). Commercially grown crops present serious issues for control as removal may not be an option, with growing cycles, harvest times, and pesticide residue limit regulations presenting significant obstacles to chemical control. Hosts that are either protected species, or growing in protected areas, such as sagelands in the Western United States, may not be controlled directly, and in such circumstances, recognition of the risk they pose and mitigation through distance may the only option.

Direct control. To date, there have been few attempts to control the phytoplasma directly because as an obligate parasite that is also fastidious and unculturable any studies must be performed in planta. In the 1970s and 1980s, multiple attempts were made to use oxytetracycline injected into trees to eliminate the phytoplasma (Amin and Jensen 1971; Lacy 1982; Nyland 1973; Sands and Walton 1975). However, while this suppressed X-disease symptoms for the current and sometimes following season depending on rate and method of application (Lacy 1982; Nyland 1973), symptoms reappeared 1 to 2 seasons after treatment was halted (Lacy 1982). Use of antimicrobials with in vitro plant cultures has been used on other phytoplasma (Tanno et al. 2018) and fastidious plant pathogenic bacteria (Zhang et al. 2011) systems, and may be viable for use against '*Ca*. P. pruni'-infected germplasm, along with screening FANA (2′-deoxy-2′-fluoro-D-arabinonucleic acid antisense) oligonucleotides that have been used with some success against citrus greening (Hunter et al. 2021; Sandoval-Mojica et al. 2021).

Planting stock certification programs. Most commercial *Prunus* species are clonally propagated, with very limited use of seed-grown plants as either rootstocks onto which desirable scion varieties are grafted, or as landscaping plants for ornamental varieties. The use of grafted plants presents a potential risk for long distance spread and introduction of the pathogen into both domestic and commercial systems, for it was recognized very early that '*Ca*. P. pruni' is readily graft-transmissible (Rawlins and Parker 1933), and nurseries are a focal point for potential infection and multiplication through their operational practices and physical setup (Richards and Cochran 1956).

The use of infected scion or rootstock material is an obvious concern, and the collection of grafting material of unknown or suspect infection status from commercial orchards is a risk that should be mediated through repeated testing before and after collection. In the United States, to mitigate this risk, there are two complimentary programs to produce and monitor pathogen-tested planting stock for and within nurseries. The first is the National Clean Plant Network (NCPN), a USDA-APHIS managed program that funds "clean plant centers" across the country whose purpose is to introduce germplasm needed by U.S. growers and producers, perform diagnostic tests for harmful and economically important pathogens, and, if positive, perform thermotherapy or meristem tissue culture to produce pathogen-free plants that are then maintained under controlled conditions and retested to ensure continued pathogen-free status (Fuchs et al. 2021). Propagative material, such as budwood or tissue-cultured plantlets, are distributed from these "clean" or "G1" plants to U.S. nurseries to establish their "mother" or "G2" blocks for further propagation (Fuchs et al. 2021). At the time of writing there are three NCPN centers that produce and distribute pathogen-tested propagative material: the Clean Plant Center Northwest at Washington State University, Foundation Plant Services at University of California-Davis, and the Clemson Clean Plant Center at Clemson University.

At the nursery level, the state departments of agriculture in California, New York, Oregon, Pennsylvania, and Washington administer certification programs to ensure the cleanliness of nursery planting stock. These certification programs (Fig. 8) track the propagation of the mother trees at the nursery from the original clean stock, and the subsequent propagation of "G3" or "G4" production stock that is sold to growers so that if a pathogen is detected, traceback can occur to identify where in the propagation system the infection occurred. At the G2 level, the relevant state departments of agriculture inspect all mother trees annually and test for select pathogens on a 2- to 3-year basis, with targeted testing for pathogens of concern if needed; the Oregon Department of Agriculture tested G2 stock in their state for '*Ca*. P. pruni' during 2021 to 2022, and the Washington State Department of Agriculture commenced doing so in 2022. At the G3 and G4 levels, visual inspection is performed with diagnostic testing applied if a pathogen is suspected.

At both the G2 and G3/G4 levels, certified blocks are to be kept at least 100 feet away from noncertified plantings, and a vector control program is to be applied. Blocks are currently permitted to be outdoors, and with no insect barriers. This contrasts with the citrus planting stock certification program in Florida (Fla. Admin. Code R. 5B-62) enacted in response to the citrus greening ('*Candidatus* Liberibacter asiaticus') epidemic in which all levels are to be propagated in enclosed, insect-proofed structures. Finally, the *Prunus* planting stock certification programs are voluntary in all five states, and participating nurseries can and do sell both certified and uncertified trees to growers, whereas in Florida certification is mandatory.

The increasing knowledge of '*Ca*. P. pruni' epidemiology suggests state nursery stock certification program protocols may need to be revised. For example, the risk of vector-mediated spread of the X-disease phytoplasma into G3 and G4 production plants should be assessed as these are currently only visually inspected. While Richards and Cochran (1956) reported that visual inspection of young peach nursery production stock was possible as infected trees were markedly smaller than their uninfected peers, sweet cherry is less sensitive to decline, and the primary symptoms appear only on the fruit. The limited efficacy of visual inspection in cherry and peach was demonstrated recently in Washington State where a sample of cherry and peach planting stock sold to growers and tested by PCR prior to planting had '*Ca*. P. pruni' incidences of up to 12% per source as determined by PCR (Harper, *unpublished data*). Phytoplasma titers were low, suggestive of vector transmission in the year prior to sale, and while of normal size and

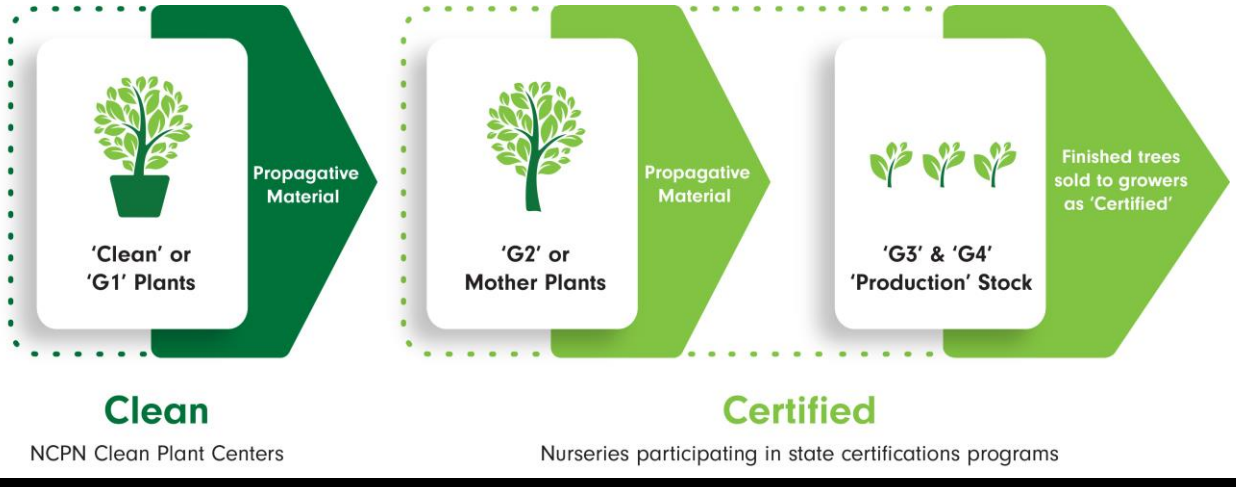

FIGURE 8
Schematic of the flow of G1 material from the NCPN clean plant centers to G2 to G3/G4 trees in nurseries participating in state nursery stock certification programs. Image courtesy of the Clean Plant Center Northwest, Washington State University, used with permission.

caliper at planting, several of the peach trees began exhibiting foliar chlorosis in the following fall (Harper, *unpublished data*).

IX. Research, Extension, and Education Priorities

The decades-long gaps between '*Ca*. P. pruni' disease epidemics in stone fruit, as well as the scattered geographic distribution, have hampered continuing research in the last century. This was most pronounced between the California outbreaks of the 1980s and the Pacific Northwest epidemic that began in the 2010s, during which time major advances in molecular techniques as well as pathogen, plant, and insect genetics were made. In this latest outbreak, comparing the literature to emerging research findings (Cooper et al. 2022; Wright et al. 2021a, b, 2022a, b) suggested that our understanding of the pathosystem as a whole was lacking, and many questions were yet to be asked, let alone answered. The knowledge gap has also hampered extension, outreach, and grower education, and thus disease mitigation and control.

RESEARCH PRIORITIES

Research priorities may be broken down into short- and mid-term needs necessary to understand and respond to an emerging outbreak, and long-term needs to prevent or reduce the likelihood of continuing economic harm:

- **Early detection and diagnosis:** The X-disease phytoplasma can be difficult to detect early in the infection cycle prior to symptom development when the pathogen concentration is low and unevenly distributed in infected trees. There is a pressing need to develop both a greater understanding of where the phytoplasma may be reliably found under these circumstances, and to develop tools with greater detection sensitivity, speed, and throughput and reduced cost relative to the PCR-based approaches used at present.
- **Influence of abiotic factors on disease progression:** Both older and more recent observations have highlighted the effect that environmental and geographic factors have on disease expression and progression throughout the course of an infection. Further research is needed to identify and determine these factors and their economic impact.
- **Effect of phytoplasma diversity on disease and spread:** There is increasing evidence of diversity within *Prunus*-infecting '*Ca*. P. pruni' isolates, and potential host or vector specificity. Understanding these relationships may be critical to projecting pathogen spread.
- **Epidemiological significance of the '*Ca*. P. pruni' host range:** The X-disease phytoplasma has a broad range of hosts beyond *Prunus* species, many of which may have epidemiological significance in the orchard and extra-orchard environment. Understanding significant hosts and the role they play in pathogen persistence and spread are relevant to developing effective control measures.
- **Host–pathogen interaction:** The interaction between the phytoplasma and host remains unknown; the disease effectors expressed by '*Ca*. P. pruni' and their targets in *P. avium* or *P. persica* need to be identified and characterized.
- **Breeding for tolerance or resistance:** The majority of *Prunus* species, both commercial and wild, are susceptible to '*Ca*. P. pruni' infection. Understanding the genetic basis for disease and developing molecular breeding tools is critical to developing tolerant or resistant commercial peach and cherry cultivars, because the pathogen is unlikely to be eradicated and the disease is likely to reappear throughout North America in the future.
- **Vector identification:** Limited knowledge of the leafhoppers that vector '*Ca*. P. pruni' in different geographic regions limits our ability to control

these insects. Studies on vector efficiency as well as placing known and unknown vectors in a phylogenetic framework may help us understand limits to vector capacity, enabling better prediction of untested vectors, and may reveal methods of disrupting vector capacity.

- **Vector–pathogen–host interaction:** Basic research on vector biology, behavior, phenology, and host plant use is required and may help uncover novel control methods to limit transmission. For example, understanding of how the phytoplasma alters vector behavior may allow development of management strategies that particularly target infective leafhoppers, and research to better understand X-disease vector genetics may allow us to develop genetic-based control methods.
- **Vector biology and ecology:** Better understanding of the ecological interactions between vectors and other insects, as well as bacterial and fungal species, is needed to identify potential biological control agents. Further research is also needed to investigate the genetic relationship between subspecies of *Colladonus*. Due to the mobile, phytophagous nature of leafhoppers, there is a need for a coordinated integrated pest management program for X-disease vectors that can be implemented areawide across multiple cropping systems.
- **Orchard removal and re-establishment:** Additional research is needed on the best practices to remove and establish an orchard, particularly assessing risk factors for infection from nursery systems.
- **Disease management in organic systems:** Organic cropping systems present significant management issues due to the absence of chemical controls of infected trees, alternate hosts, and leafhopper vectors. Developing effective control measures based on vector and pathogen biology is essential to the sustainability of organic systems.

EXTENSION AND EDUCATION PRIORITIES

Extension outreach and education is a vital part of disease awareness and management. Short- and mid-term goals need to be focused on the current outbreak of little cherry disease. Long-term goals should be more focused on continuous education and best management practices to help mitigate future disease outbreaks.

- **Increase awareness of the distribution and importance of X-disease:** Unified messages need to be presented to all industry members, from growers to packers and lobbyists on the distribution, incidence, and impacts of X-disease. Emphasis should be placed on the economic risks and harms of X-disease as the most tangible and relatable message to all sectors of the stone fruit industry.
- **Continued education on disease biology:** Individual orchardists have experienced X-disease at different levels of pressure, rates of progression, and severities in their orchards, and may dismiss the threat of the pathogen. Continuing education on disease symptoms, progression, transmission, and pathogen host range is needed, and messaging must be consistent, with an emphasis on the changing nature of research findings and translation of evolving research data into tangible facts for the growers. Education is important to allow growers to place field scouting and diagnostic test results in context.
- **Outreach to small, under-resourced and less-aware stakeholders:** Direct outreach from extension agents and crop consultants has been effective in reaching larger growers. However, more emphasis is needed to reach small/hobby growers and homeowners with a few backyard trees. Extension professionals need to target groups of stakeholders through more varied and diverse means, not only in industry meetings and through county pest boards, but with Master Gardener and 4-H groups as well. Media campaigns need to be calibrated to reach different sections and age groups within the grower community. Traditional media

such as radio, newspapers, and industry publications should be complemented with targeted messaging and tools on social media to reach younger producers and crop consultants. Given the diverse agricultural community, all messaging must be in both English and Spanish.

- **Emphasize tree removal as critical to disease management:** Tree removal remains the single most effective means of reducing inoculum load and reducing disease pressure. However, many growers are still reluctant to remove trees that are lightly infected, and/or allow orchards to get progressively more infected until they are no longer harvestable. Demonstrating the epidemiological importance of tree removal should be partnered with financial incentives to do so. Advocacy for tree-removal policy, legislation, and enforcement may be required for area-wide management.
- **Vector identification and management:** Identification of leafhoppers and sustainable management options are needed for both conventional and organic growers. Management practices should include pesticide applications and horticultural practices such as exclusion, reflective materials or deterrents, and vector host management.
- **Increase knowledge of alternative hosts and their management:** There is a need to increase grower knowledge of which plants are alternative (non-*Prunus*) hosts for X-disease and leafhopper vectors and options for management of these plants including cultural controls, broadleaf herbicides, and optimal timing and rotation of herbicides.
- **Increase knowledge of best management practices for new plantings:** There is a need to develop and promote best management practices for effectively replanting orchards, including replanting intervals, effective insecticide use, sourcing pathogen-tested planting stock, and risk management while replanting.
- **Best management practices for nurseries:** Nurseries are a significant contributor to the spread of X-disease, and the development and adoption of best management practices consistent with up-to-date knowledge of pathogen and vector biology are needed to ensure the cleanliness of nursery stock and ensure grower confidence.
- **Assess and integrate new products and technologies:** New diagnostic tools and platforms, area-wide mapping, and prediction tools that integrate vector, pathogen and environmental models are needed to inform and educate growers.

X. Infrastructure and Experts

The following personnel have in-depth knowledge and/or experience with the X-disease pathosystem in the United States:

Pathology

Scott Harper – Department of Plant Pathology, Washington State University, Prosser, WA; scott.harper@wsu.edu

Jay Pscheidt – Department of Botany and Plant Pathology, Oregon State University, Corvallis, OR; pscheidj@science.oregonstate.edu

Madalyn Shires – Department of Agronomy, Horticulture & Plant Science, South Dakota State University, Brookings, SD; madalyn.shires@sdstate.edu

Entomology

W. Rodney Cooper – Temperate Tree Fruit and Vegetable Research Unit, USDA-ARS, Wapato, WA; rodney.cooper@usda.gov

Tobin Northfield – Department of Entomology, Washington State University, Wenatchee, WA; tnorthfield@wsu.edu

Extension

Tianna DuPont – Tree Fruit Extension, Washington State University, Wenatchee, WA; tianna.dupont@wsu.edu

Mohamed Nouri – Cooperative Extension San Joaquin County, University of California, Stockton, CA; mnouri@ucanr.edu

Bernardita Sallato – Tree Fruit Extension, Washington State University, Prosser, WA; b.sallato@wsu.edu

Ashley Thompson – Fruit Tree Extension Wasco and Hood River Counties, Oregon State University, The Dalles, OR; ashley.thompson@oregonstate.edu

XI. Web Resources

General Disease Information

Michigan State University: https://www.canr.msu.edu/ipm/diseases/x-disease

Pacific Northwest Pest Management Handbook: https://pnwhandbooks.org/plantdisease/host-disease/cherry-prunus-spp-x-disease

University of California: https://www2.ipm.ucanr.edu/agriculture/cherry/X-disease-cherry-buckskin/

Washington State University: http://treefruit.wsu.edu/crop-protection/disease-management/western-x/

Disease and Vector Identification Guides

Symptom Gallery (WSU): http://treefruit.wsu.edu/crop-protection/disease-management/western-x/symptoms-gallery/

Cherry Symptom Video: http://treefruit.wsu.edu/videos/symptoms-of-little-cherry-virus-and-x-disease-phytoplasma/

Stone Fruit Symptom Video: http://treefruit.wsu.edu/videos/symptoms-of-x-disease-phytoplasma-in-stone-fruit/

Scouting and Sampling Guide (WSU): http://treefruit.wsu.edu/crop-protection/disease-management/western-x/sampling-guide/

Vector Gallery (WSU): http://treefruit.wsu.edu/vector-gallery/

Vector Identification and Trapping Video (WSU): http://treefruit.wsu.edu/videos/x-disease-vector-management/

Replanting and Recovery Information

USDA-FSA Tree Assistance Program: https://www.fsa.usda.gov/Assets/USDA-FSA-Public/usdafiles/FactSheets/tree_assistance_program-tap-fact_sheet.pdf

Nursery Best Management Practices (WSU): http://treefruit.wsu.edu/nursery-prevention-lcd/

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