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Genetic and Molecular Disease Management of Powdery Mildew, Bacterial Canker, and X-Disease in US Pacific Northwest Sweet Cherry: Current Obstacles and Future Opportunities

ALEXANDRA M. JOHNSON[1], LYNDON D. PORTER[2], GARY GROVE[3], SCOTT J. HARPER[3], CAMERON P. PEACE[1]

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Abstract

The Pacific Northwest produces most sweet cherry fruit for the United States, which is second to Turkey in global sweet cherry production; however, yield is impeded by infection-incurred losses. Endemic pathogens significantly limiting production include *Podosphaera cerasi* causing powdery mildew, *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringae* pv. *morsprunorum* races 1 and 2 causing bacterial canker, and '*Candidatus Phytoplasma* pruni' causing X-disease. While significant resources are annually spent to manage these pathogens, use of disease resistant cultivars as well as an understanding of the underlying genetic and molecular mechanisms involved in plant defense responses might facilitate better infection management solutions. In particular, identification of genes responsible for conferring resistance to these pathogens and then combining resistance alleles into new sweet cherry cultivars offers a sustainable solution for disease management.

Overview of Sweet Cherry Production in the Pacific Northwest

Sweet cherry (*Prunus avium* L.) is a highly valuable rosaceous crop second only to apple (*Malus domestica* Borkh.) in terms of economic significance in many temperate regions (Noorazar et al., 2020; USDA-NASS, 2022), yet production is hindered by fungal and bacterial pathogens (Mgbechi-Ezeri, 2016 ; Molnar et al., 2022; Olmstead et al., 2000). The largest global producer of sweet cherry is Turkey, with 860,000 metric tonnes produced in 2021 (USDA-FAS, 2021), followed by the United States (US), which produced 372,000 tonnes over 34,196 hectares (84,500 acres) at a value of $866 million in 2021 (USDA-NASS, 2022). In the US, the largest proportion of sweet cherries are grown in the Pacific Northwest (PNW), which in 2022 encompassed 20,437 hectares (50,500 acres) of orchards in the states of Washington and Oregon and produced 280,000 tonnes of fruit (USDA-NASS, 2022). However, not all cherry fruit is harvestable or marketable, and while no information is available regarding tonnes of fruit left unharvested (Hanrahan, I., personal communication), 6,350 tonnes of unsold cherry were reported for 2021 (USDA-NASS, 2022). The most significant hindrance to sweet cherry production stems from disease-incurred loss (Galinato et al., 2019; Molnar et al., 2022). Topically applied, chemical treatments for fungal and bacterial diseases can be contact only, locally active, or systemically diffused throughout plant tissues, but these applications are only effective against genetically susceptible pathogens (Crosse and Garrett, 1958; Hubbard and Probst, 2017). Both fungal

[1] Department of Horticulture, Washington State University, Pullman, WA 99164
[2] U.S. Department of Agriculture, Agricultural Research Service, Grain Legume Genetics and Physiology Research Unit, Prosser, WA 99350
[3] Department of Plant Pathology, Washington State University, Prosser, WA 99350

[1] To whom reprint requests should be addressed. Email address: alexandra.johnson2@wsu.edu; cpeace@wsu.edu

and bacterial infections can occur when pathogens overcome physical or biochemical resistance barriers, or be opportunistic, as is found in *Monilinia spp*. fungi which colonize fruit that have had their epidermis compromised by moisture-induced cracking (Quero-Garcia et al., 2019). Infections that become systemically established become far more difficult and costly to treat, and some diseases might not be treatable, which may ultimately result in tree removal as the best and most economical solution (Harper et al., 2020; Van Steenwyk et al., 1995). Furthermore, infections in sweet cherry elicited by multiple pathogens are common, requiring complicated solutions for preventing crop loss (Abdullah et al., 2017; Murray and Jepson, 2018). Elucidating the underlying mechanisms governing infection establishment and disease progression could therefore be valuable in devising improved treatment and management strategies to increase production (Dean et al., 2012).

While much attention has been given to development of synthetic, antimicrobial chemistries to combat diseases in sweet cherry, some plants have been identified to be less susceptible to completely resistant to infection from some pathogens. With the rise in resistance from fungal (Hubbard and Probst, 2017) and bacterial (Claflin, 2003) pathogens to certain synthetic chemistries, successful efforts have been made to identify cherry cultivars that can naturally, genetically resist infection from certain pathogens (Mgbechi-Ezeri, 2016; Olmstead et al., 2000). Plant-host-derived resistances for powdery mildew and bacterial canker diseases have been identified; however, resistances have not yet been found for all economically important pathogens affecting sweet cherry (Mgbechi-Ezeri, 2016; Olmstead et al., 2000).

In recent years, development of cultivars that resist or at least tolerate infection has risen among the list of priorities for breeding efforts to combat infection-incurred losses (Gallardo et al., 2012). In addition to producing most of the domestic fresh market cherries, the PNW is also a significant developer of new sweet cherry cultivars (Oraguzie et al., 2017; USDA-NASS, 2020). Within Washington state, the Pacific Northwest Sweet Cherry Breeding Program of Washington State University exists to meet the need for locally adapted cultivars, particularly those that can resist infection from pathogens (Oraguzie et al., 2017). Assessment of underlying genetics in cherry trees for resistance to the endemic PNW pathogens that cause powdery mildew, bacterial canker, and X-disease and then exploiting disease resistance via breeding efforts to create new cultivars with improved resistance could offer an economically efficient solution to addressing crop loss (Mgbechi-Ezeri, 2016; Olmstead et al., 2000; Quero-Garcia et al., 2017; Van Steenwyk et al., 1995). In addition, to the extent that genetic investigation confirms or reveals genetic resistance levels among existing cultivars, it could inform appropriate cultivar choice for growers.

Fungal Infection Impacting Cherry Production

Powdery mildew.

The fungal pathogen *Podosphaera cerasi* [formerly known as *Podosphaera clandestina* (Wall. Fr.) Lev., revised in Moparthi et al., 2019] is the causal agent of powdery mildew of sweet cherry. In the absence of fungicides or host genetic resistance, the disease occurs annually, and symptoms usually include visible blemishes or gray to white lesions on both leaves and fruit (Olmstead and Lang, 2002). Provided adequate spring moisture between bud break and pit hardening, the rigid, spherical perennation structures of *P. cerasi* known as chasmothecia open and release ascospores (Grove and Boal, 1991a and 1991b), the only known source of primary inoculum. Primary infection from fungal ascospores begins in early- to mid-spring in physiologically and genetically vulnerable plant tissues (Webster and Webber, 2007). After establishment and in later stages of infection, aerial mycelial extensions known as hyphae develop and generate concatenated strands of conidia (Fig. 1), which are asexual spores that serve to spread

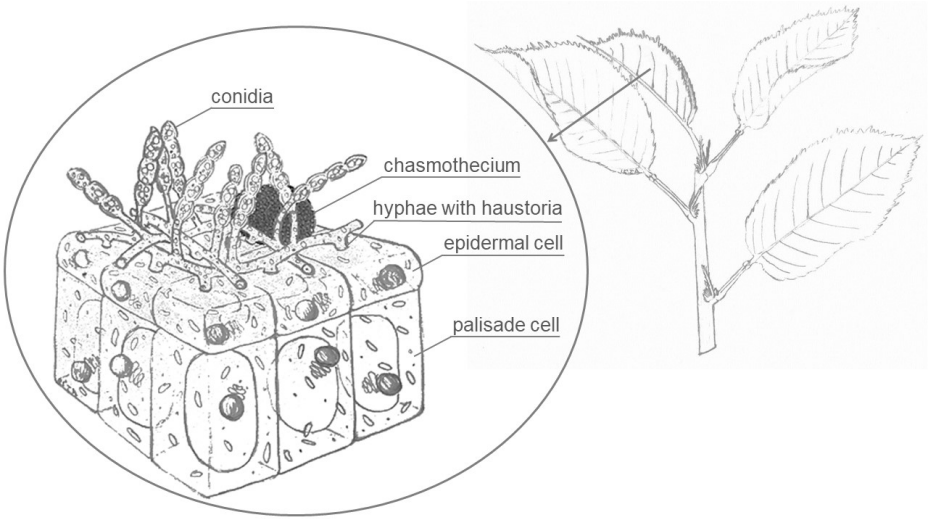

Fig. 1 Illustration of established powdery mildew (*Podosphaera cerasi*) infection in sweet cherry (*Prunus avium*) leaf epidermal cells including infectious, concatenated conidia, and a chasmothecium. Early infections are localized to the abaxial side of expanding leaves.

the pathogen (Webster and Webber, 2007).

In foliage, primary fungal infections that begin in early- to mid-spring give rise to secondary infections that can occur throughout the growing season. Ascospores first establish primary infections in susceptible emerging leaves that have not fully expanded as well as in young stems (Grove and Boal, 1991a and 1991b; Olmstead et al., 2000). Primary infections are typically few in number and confined to foliage originating directly from scaffold limbs or near tree crotches, or both. Secondary infections are typically first observed on the third leaf from shoot apices beginning in mid- to late-May in Eastern Washington (Grove and Boal, 1991b). At this growth phase, leaf epidermal cells are particularly vulnerable to infection, because they are soft and have yet to develop a mature cuticle, which is the initial barrier to infection (Evert, 2006). Leaves damaged by infection have reduced photosynthetic capabilities and thus trees with powdery mildew disease have reduced vigor (Grove and Boal, 1991b; Olmstead et al., 2000). In May, wind disperses conidia to neighboring sweet cherry trees (Grove and Boal, 1991a and 1991b). If those trees are also genetically susceptible and the tissues physiologically susceptible, the spores germinate and perpetuate the repeating cycle that can persist until late Aug. (Grove and Boal, 1991a and 1991b).

While foliar powdery mildew reduces tree vigor, the most consequential economic impact occurs when the pathogen infects fruit. Infection of sweet cherry fruit by *P. cerasi* occurs in much the same way as leaf infection, with the pathogen remaining localized to epidermal cells (Olmstead et al., 2000; Webster and Webber, 2007). Once infected, the presence of mycelia damages fruit cosmetically and, as the disease progresses, fruit are prone to degradation and decay (Murray and Jepson, 2018; Olmstead et al., 2000). Observably blemished and deteriorating fruit are not saleable, and instead, result in an economic loss (Hanrahan, I., personal communication). Beyond a domestic market issue, the pathogenic fungi can remain interstitially viable and resistant to fungicidal sprays, and are therefore a problem in the nursery trade when growers

inadvertently purchase and then plant infected plants (Swamy et al., 2019).

Powdery mildew management has historically relied on the physiological strategies of canopy management and application of fungicidal sprays, but with drawbacks. Because lack of light and air movement promotes fungal infection establishment, pruning branches to increase both of these factors within the canopy reduces powdery mildew incidence (Calabro et al., 2009). However, pruning must be repeated throughout the year to maintain canopy structure and thus can be costly (Calabro et al., 2009; Hubbard and Probst, 2017). With fungicidal sprays, a major problem is the efficacy of an applied fungicide to control disease often diminishes over the multi-year duration of its use (Colucci et al., 2008; Hubbard and Probst, 2017), requiring constant attention by scientists to develop new fungicide chemistries (Vielba-Fernández et al., 2020). Additionally, application of fungicides can significantly alter orchard microbial community dynamics and exert artificial selective pressure on fungal pathogens that can favor development of fungicidal-resistant types (Loland and Singh, 2004). Further alterations or damage to biological community structures can occur when sprayed fungicides or their residues contaminate soil and groundwater (Bedos et al., 2010; Nettles et al., 2016). Such off-target effects resulting from accidental misapplication or off-site movement have led to marked changes in orchard ecosystems (Loland and Singh, 2004). Therefore, rather than exclusive reliance on pruning or sprayed products, powdery mildew management might be best achieved via an integrated approach that also encompasses host genetic resistance (Olmstead and Lang, 2002; Dreistadt, 2016).

While most sweet cherry cultivars grown commercially are susceptible to infection from powdery mildew, certain cultivars and selections exhibited reliable disease resistance (Olmstead et al., 2000 and 2001; Olmstead and Lang, 2002). The first cherry tree to be recorded as mildew resistant was a chance seedling found growing near Prosser, WA, by the Washington State University sweet cherry breeder, Dr. Thomas Toyama (Toyama et al., 1993). This mildew-resistant seedling was named Powdery Mildew Resistant-1, or PMR-1 (Toyama et al., 1993). Inheritance analyses revealed a single allele with a dominant effect was responsible (Olmstead et al., 2001), named powdery mildew resistance factor-1, or *Pmr1* (Olmstead and Lang, 2002). The resistance-associated *Pmr1* allele appears to be capable of conferring powdery mildew resistance in foliar as well as fruit tissues and pedigree analysis of mildew resistant cherry plants indicates other, *Pmr1*-like alleles might be present (Peace et al., 2018). Recent pedigree analysis for PMR-1 revealed it to be the offspring grandchild of the cultivar ‘Moreau’ (Demir, 2019), which has also independently been identified as phenotypically resistant to mildew infection and carrying the *Pmr1* allele that PMR-1 inherited from it (Peace et al., 2018). ‘Chelan’, an offspring of ‘Moreau’, is also phenotypically resistant to mildew infection and inherited the *Pmr1* allele from ‘Moreau’ (Olmstead and Lang, 2002; Demir, 2019). Examination of diverse breeding germplasm of Washington State University determined other cultivars, advanced selections, and wild crop relatives are resistant to mildew infection, shown in Table 1 (Olmstead et al., 2001; Peace et al., 2018; Zhao, 2013). Whole-genome genotypic profiling of single nucleotide polymorphisms (SNPs) using a 6K SNP array developed for cherry (Peace et al. 2012) genetically mapped the *Pmr1* locus to the proximal end of chromosome 5 (Zhao, 2013; Demir, 2019).

While the underlying mechanism governing genetic resistance to fungal infection has not been fully explored in sweet cherry, preliminary assessment in sweet cherry’s more economically valuable relatives, peach [*Prunus persica* (L.) Batsch] and apple have provided some insight. Resistance to infection establishment for both *Prunus* and *Malus* was reported to be the product of a pathogen-associated recognition cascade, culminating in programmed death of the affected cell (Al-

Table 1. List of sweet cherry cultivars previously described as resistant or susceptible to powdery mildew (*Podosphaera cerasi*). The cultivar Venus has been described as susceptible to mildew infection by Olmstead et al. (2001) and resistant by Peace et al. (2018).

Resistant	Susceptible
Chelan	Ambrunes
Cristobalina	Bing
Early Burlat	Lapins
Hedelfinger	Rainier
Mildew-Immune Mazzards	Selah
Moreau	Sunburst
PMR-1	Van
Regina	Venus
Sato Nishiki	
Schneiders	
Venus	

magro et al., 2008). The gene products implicated in eliciting this programmatic cellular response are a suite of receptors belonging to a gene family characterized as encoding for nucleotide-binding site leucine-rich repeats (DeYoung and Innes, 2006; McHale et al., 2006; Feng et al., 2019; Zhong et al., 2022). Once receptors are triggered, host cell death prevents pathogen establishment and thus halts infection establishment and disease progression (McHale et al., 2006). Because of the phylogenetic proximity of *M. domestica* and *P. persica* to *P. avium* within the family Rosaceae, future molecular physiological and genetic research with these related species might uncover additional similarity in the fungal resistance mechanism present in sweet cherry.

Another form of mildew resistance reported in apple that works via reducing susceptibility might also be present in sweet cherry. Within apple, several genes associated with plant immunity regulation become downregulated upon infection from *Podosphaera leucotricha*, the cause of powdery mildew in this plant species (Pessina et al., 2014). This downregulation results in disease resistance via reduction of susceptibility. Plant resistance to mildew infection is achieved by alleles that impair susceptibility at several *Mildew Locus O* (*MLO*) loci, which are conserved across several plant taxa (Pessina et al., 2016). Genome-wide association studies using *MLO* sequence data gathered from apple (Pessina et al., 2016) identified homologous regions within the sweet cherry genome (Jiwan, 2011; Kenta et al., 2017); however, more work is needed in the future to determine if the functional resistance mechanism in apple is also functional in cherry.

Bacterial Infections of Sweet Cherry in the Pacific Northwest

Bacterial canker.

Bacterial canker disease in sweet cherry is caused by *Pseudomonas syringae* pv. *syringae* or *Pseudomonas syringae* pv. *morsprunorum* race 1 and 2 (Mgbechi-Ezeri et al., 2018). This disease can result in up to 50% yield loss as well as 75% tree mortality (Puławska et al., 2017; Spotts et al., 2010b). Unlike fungal infection from the powdery mildew pathogen *P. cerasi, P. syringae* infections in trees can occur

at any time and are not limited by organ type (roots, stems, leaves, inflorescence, or fruit) because all plant organs can develop disease (Mgbechi-Ezeri et al., 2017). Furthermore, once bacteria-causing cankers become established in the plant's vasculature, infection has the potential to proliferate systemically (Otto et al., 2018), which poses a threat to orchard establishment and production longevity (Farhadfar et al., 2016). Greater virulence in sweet cherry has been reported from infection by *P. syringae* pv. *syringae*; however, both *P. syringae* pathovars are capable of disease-induced mortality (Mgbechi-Ezeri et al., 2017).

P. syringae bacteria can initially gain entry and establish infection in cherry plants through plant injury, with frost damage and inadvertent inoculation from pruning implements being the most common causes (Moore, 1988; Spotts et al., 2010b). However, of significant epidemiological consequence is the ability of *P. syringae* to spread via wind and establish infection through open stomata (Fig. 2), with the plant's vascular channels subsequently transformed into bacterial thoroughfares (Xin et al., 2018). While the bacteria might initially reside asymptomatically, localized infections found in early stages of bacterial canker disease can appear as "blossom blast" (indicated by dead, black calyces in floral buds that fail to open) and "stem dieback" (observed as rapid wilting and subsequent death of young stems), while systemic infections can include necrosed lesions with gummosis on trunks and branches (Moore, 1988; Spotts et al., 2010a). Prolonged damp conditions are favorable for all stages of pathogenesis because moisture saturation reduces plant host vigor while facilitating pathogen mobility via splash dispersal (Moore, 1988; Petriccione et al., 2017). As such, wet seasons tend to be the time for most transmission and new infection (Kennelly et al., 2007; Spotts et al., 2010a). Additionally, because *P. syringae* is a generalist pathogen and capable of asymptomatic colonization of a range of plant hosts, it can become an endemic threat if present in weeds on the orchard floor as well as in shrubs used as fence rows adjacent to cherry orchards (Kennelly et al., 2007).

Control of *P. syringae* populations in

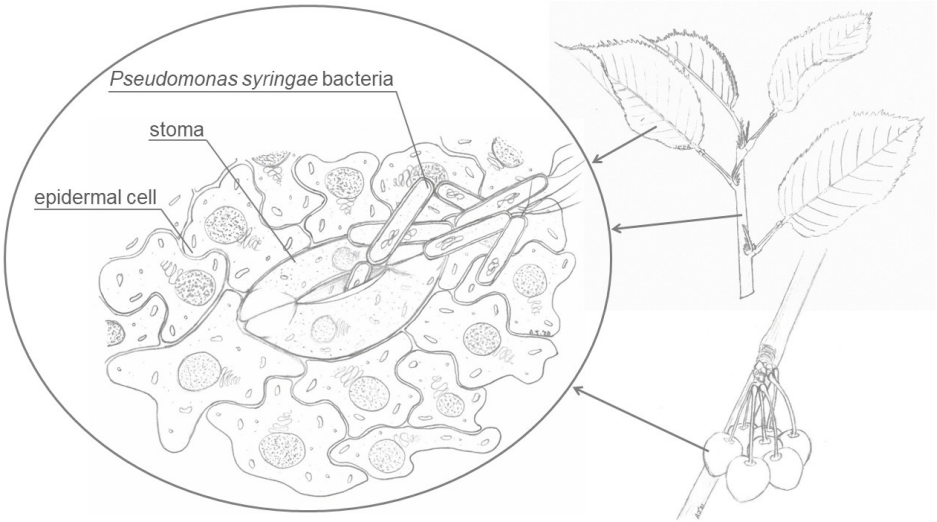

Fig. 2. Illustration of *Pseudomonas syringae* bacteria entering an open epidermal stoma. Open stomata on young, vegetative tissues including leaves, stems, and fruit can be colonized.

cherry orchards has historically relied upon the physiological strategy of orchard application of sprayed copper compounds and, more recently, antibiotics (Claflin, 2003), as well as canopy and orchard floor management to remove sources of inoculum (Spotts et al., 2010a). However, the efficacy of cultural and chemical bacterial canker control has remained low, likely due to colonized but non-diseased, non-sweet cherry plants serving as pathogen reservoirs within and adjacent to orchards (Kennelly et al., 2007). Additionally, chemical control puts selective pressure on pathogen strains and increases the likelihood of only highly virulent pathovars surviving, thereby altering pathogen community structure (Claflin, 2003). The presence of endemic, virulent strains of *P. syringae* in sweet cherry orchards of the PNW has prompted research into devising other means of mitigating bacterial canker infection – in particular, identifying genetic sources of host resistance among sweet cherry cultivars (Bedford et al., 2002; Mgbechi-Ezeri et al., 2017).

While genetic sources for host resistance to bacterial canker infection have been reported in sweet cherry, the evidence regarding which cultivars are resistant and which are susceptible has been partially conflicting. The production-leading cultivars 'Bing' and 'Sweetheart' have been universally reported to be susceptible to infection (Bedford et al., 2002; Junior, 2000; Mgbechi-Ezeri et al., 2017; Spotts et al., 2010a, 2010b). Cultivars 'Rainier' and 'Regina' have been identified as resistant to bacterial canker infection (Spotts et al., 2010b), yet other reports list 'Rainier' and 'Regina' as susceptible, while 'Early Burlat', 'Lambert', and 'Corum' were indicated as being resistant (Junior, 2000). Host resistance has also been reported in the selection PMR-1 and several of its offspring (Mgbechi-Ezeri et al., 2017). Inconsistent resistance vs. susceptibility results might be due to different bacterial strains, a limited number of cultivars compared, a limited number of individual plants observed for each cultivar, differential interactions among rootstocks and scions, or macroclimate or microclimate differences affecting the quality of plant material used for testing at the time of inoculation (Beckman et al., 2002; Junior, 2000; Mgbechi-Ezeri, 2016; Mgbechi-Ezeri et al., 2017; Spotts et al., 2010b). Therefore, increasing the number of genetically unique trees assessed with sufficient replication of each and standardized experimental conditions would be expected to provide clarity regarding cultivar differences for this trait by minimizing confounding external factors. Furthermore, evaluation over a recorded range of growing conditions and locations and encompassing various *P. syringae* strains should help identify host resistance differences among cultivars by accounting for confounding external factors (Spotts et al., 2010b).

Recent technological advances in genomics have facilitated the association of phenotypic traits with underlying genetic factors, which could be employed to identify host resistance alleles for bacterial canker and the sweet cherry plants harboring those alleles (Mgbechi-Ezeri et al., 2018). Because bacterial canker resistance appears to have a significant genetic component, identification of alleles and loci involved in bacterial canker resistance inheritance and characterizing their phenotypic effects would provide useful information for breeding resistant sweet cherry cultivars (Mgbechi-Ezeri, 2016). Alleles associated with resistance to infection from *P. syringae* have been documented in other plant species, including the model *Arabidopsis thaliana* and in sweet cherry's close relative, apricot (Omrani et al., 2019; Xin et al., 2018). In apricot, two alleles associated with resistance to *P. syringae* were discovered and putatively identified as components in the abscisic acid pathway (Omrani et al., 2019). In the *A. thaliana* model, it was reported that plant-mediated immunity was negatively regulated by the presence of a protein, RIN4 (resistance to *P. syringae* pv. *maculicola* 1-interacting protein). The localized presence of RIN4 on plasma membranes was reported to negatively regulate stomatal closure and formation of callose plugs; however, upon effector triggering from

Pseudomonas, RIN4 is cleaved and an immune response is triggered (Xin et al., 2018). Genes found in *A. thaliana* to be specific to *P. syringae*-infection resistance might also be present and potentially similarly active in other plants affected by *P. syringae* infection such as sweet cherry. Sequence comparison of the sweet cherry genome with the *A. thaliana* genome indicates *RIN4*-like genes are present (Jung et al., 2018). Future research to determine if the molecular mechanism observed in *A. thaliana* is also functional in sweet cherry would be useful, particularly if specific alleles or allelic combinations can be identified as responsible for conferring reduced infection response or infection resistance. These alleles and their germplasm sources could then be targeted by breeders to develop new cultivars capable of growing disease-free in regions known to harbor *P. syringae*.

X-Disease.

A greater immediate and long-term bacterial threat than bacterial canker is infection from the soft-bodied pathogen '*Candidatus* Phytoplasma pruni' (*Ca*. P. pruni) that causes X-disease. Most members of Kingdom Bacteria, including bacterial canker-causing *P. syringae*, contain a cell wall composed of cross-linked peptidoglycans; however, phytoplasmas such as *Ca.* P. pruni are physiologically distinct and lack a peptidoglycan wall structure, making them appear pleiomorphic microscopically (Razin, 2006). Instead, phytoplasmas have a single cell membrane and form a distinct genus within the bacterial class *Mollicutes* (Hogenhout et al., 2008; Razin, 2006). Phytoplasmas are obligate intracellular pathogens; only the sieve tube elements of plant hosts or hemolymph of insect vectors are suitable for *Ca*. P. pruni survival (Davis et al., 2013; Fiore at al., 2018; Uyemoto and Kirkpatrick, 2011). *Ca*. P. pruni phytoplasmas are spread from infected to non-infected cherry trees by polyphagous leafhopper insects (family Cicadellidea) that acquire and transmit the pathogen while feeding on sap from the phloem (Davis et al., 2013; Fiore et al., 2018). Phytoplasmas transmitted during feeding can then multiply in sieve tube elements and diffuse through sieve plates to establish infection throughout the tree vasculature (Fiore at al., 2018; Uyemoto and Kirkpatrick, 2011), as shown in Fig. 3.

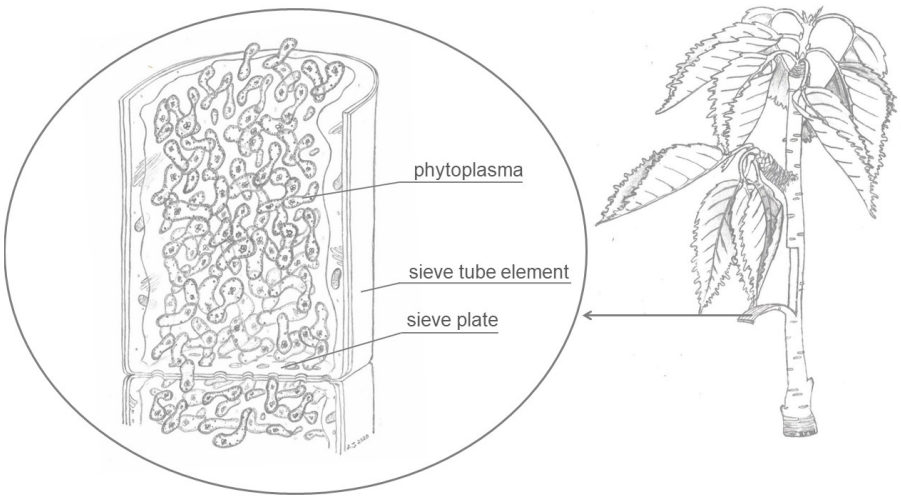

Fig. 3. Illustration of '*Candidatus* Phytoplasma pruni' infesting a sweet cherry (*Prunus avium*) phloem sieve tube element.

Phytoplasma infection in cherry is chronic, systemic, and culminates in plant death within 8-10 years, yet visible symptoms of disease can be localized, ephemeral, and myriad, which has earned this condition the name X-disease (Harper, S., personal communication; Van Steenwyk et al., 1995; Wright et al., 2021). Symptoms of X-disease first appear in fruit and include delayed color development as the fruit matures, reduction in sugar and secondary metabolites followed by reduction in size, alteration of cultivar-specific morphology (e.g., rounded fruit becoming pointed), and reddening of suture lines in certain cultivars (James et al., 2017; Uyemoto and Kirkpatrick, 2011; Van Steenwyk et al., 1995; Wright et al., 2021). The severity of fruit symptoms can vary among individual fruit within the same cluster as well as among clusters throughout the tree (James et al., 2017; Uyemoto and Kirkpatrick, 2011). Other observable symptoms of infection that can appear during later stages of infection include overall reduction of foliage and eventual decline of the tree (James et al., 2017; Uyemoto and Kirkpatrick, 2011).

Controlling X-disease in cherry has relied on the physiological strategy of maintaining sanitary practices in the orchard to prevent infections, such as repeated spraying for insect vectors, coupled with observational monitoring to detect symptoms of disease and then removal of infected trees (Harper et al., 2020). Antibacterial treatments have been proven to be both expensive and impractical (Tanno et al., 2018). As with all Mollicutes, traditional antibacterial compounds such as those in the β-lactam or glycopeptide classes are ineffective against *Ca*. P. pruni because the lack of peptidoglycan cell walls renders them impervious to chemical control compounds that rely on inhibition of peptide cross-linking and cell wall formation as the mode of action (Bertaccini, 2007; Maniloff , 2002). Even direct injection with 6-8 L per tree of the broad-spectrum antibiotic tetracycline, which is in a different antibiotic class and does not target cell walls but instead acts on the 30S ribosomal subunit to allosterically inhibit translation, has been reported to have little effect against phytoplasma infection (Bertaccini, 2021; Sands and Walton, 1975; Tritton, 197). Little efficacy in mitigating phytoplasma infections has also been demonstrated for copper-based sprays (Faramarzi et al., 2018). Unlike other bacteria such as *P. syringae* that can be treated with copper and antibiotic sprays and can also be easily cultured in laboratories (Mgbechi-Ezeri et al., 2013 and 2018), phytoplasmas are environmentally fastidious and have historically been difficult to study outside of their host plant's active growing season (Contaldo et al., 2016). Additionally, *Ca*. P. pruni has an extensive host range including the North American native relative of sweet cherry, chokecherry (*P. virginiana*), and can persist near orchards in numerous plant hosts (Uyemoto and Kirkpatrick, 2011; Wright et al., 2021). The pathogen can also persist in orchards even after trees are removed if living infected root material is still present in the soil (Davis et al., 2013; Wright et al., 2021). The primary control method for X-disease currently is disruption of the infection transmission cycle via repeated applications of insecticidal sprays to reduce the presence of the leafhopper vector; however, this method is not always effective (Davis et al., 2013). The other common yet drastic control option in use for sweet cherry commercial production is removal of diseased trees that would otherwise be infection reservoirs (Harper et al., 2020; Van Steenwyk et al., 1995).

Information regarding genetic resistance in sweet cherry to phytoplasma infection is scant. Evidence has been reported for sweet cherry cultivar-specific responses to phytoplasma infection, including differences in disease progression rate and level of symptom expression under certain environmental conditions (Wright et al., 2021), but host resistance has not been reported. The underlying genotypic differences governing the variability in response to phytoplasma infection among cultivars grown under different environmental conditions that affect symptom progression has yet to be elucidated (Wright et al., 2021).

Also unknown is if any rare cherry cultivars or wild crop relatives are genetically capable of resisting or tolerating infection from *Ca*. P. pruni asymptomatically – as has been reported in other plant species (Davis et al., 2013; Uyemoto and Kirkpatrick, 2011; Van Steenwyk et al., 1995; Wright et al., 2021).

Research to understand the phytoplasma infection mechanism, develop effective chemistries to prevent X-disease in the short-term, and elucidate the molecular, physiological, and genetic control of symptom manifestation to devise long-term solutions is hindered by the inability to experiment directly on the pathogen year-round, free from an insect or plant host (Bertaccini, 2007). The only current strategy for studying the X-disease pathogen has been maintenance of infected plants or infected leafhopper vectors, which is difficult and costly (Ambrožič-Dolinšek et al., 2008; Tanno et al., 2018). Promisingly, isolation and maintenance of *Ca*. P. pruni via *in vitro* culture appears possible because phytoplasma culturing has been previously documented for a diversity of isolates from a wide range of infected plant crops including grape (*Vitis vinifera* L.), periwinkle (*Catharanthus roseus* L.), cassava (*Manihot esculenta* Crantz), and sugar cane (*Saccharum officinarum* L.) (Álvarez et al., 2017; Contaldo et al., 2016). Therefore, future research dedicated to isolation and *in vitro* maintenance of *Ca*. P. pruni would be useful for improving access to studying this pathogen directly, outside of traditional hosts, and for efforts dedicated to developing specific orchard spray chemistries for combatting infection.

Future Prospects

Resisting establishment of pathogen infection in sweet cherry is a prime target for improving fruit yield and quality while reducing tree management expenses, ecological consequences from pesticide applications, and the threat of losing orchard trees. While many management solutions exist for commercial orchards in the form of short-term cultural practices (MacHardy, 2000) and antimicrobial sprays (Claflin, 2003; Hubbard and Probst, 2017), effective sustained control could be enhanced by exploiting genetic (i.e., heritable) resistance within the sweet cherry host itself (Quero-García et al., 2019). There are several viable approaches to elucidating the genotypic differences associated with resistance, reduced susceptibility, asymptomatic tolerance, and susceptibility to PNW pathogens in sweet cherry. Comparative transcriptomics was used to successfully identify differentially expressed genes involved in resistance pathways to a fungal pathogen in apple (Feng et al., 2019). Another molecular physiological approach is "reverse genetics", ascertaining roles of specific genes and causal effects of their alleles or lack of presence via genetic engineering, as successfully demonstrated for fusarium head blight caused by the fungus *Fusarium graminearum* in wheat, *Triticum aestivum* (Soni, 2021). The "forward genetics" approaches of QTL analysis and association mapping have been widely used, including in sweet cherry, to identify loci and their functional alleles segregating in germplasm (Iezzoni et al., 2020). Once desirable alleles for disease resistance are identified in sweet cherry germplasm, they could be incorporated into breeding populations and tracked to breed new cultivars (Iezzoni et al., 2020).

The availability of "resistance alleles" from several germplasm sources, each presumably associated with a different genetic mechanism to mitigating a disease (Sun et al., 2017), would be advantageous in breeding (Iezzoni et al., 2020). "Pyramiding" such genetic factors into single individuals via breeding to achieve enhanced disease resistance has been demonstrated with success in other crops related to sweet cherry such as peach, strawberry, apple, and pear (Lasserre-Zuber et al., 2018; Iezzoni et al., 2020), as well as in potato and corn (Knaus et al., 2019; Ullstrup, 1972), but has yet to be fully realized in cherry (Baumgartner et al., 2015; Lasserre-Zuber et al., 2018). For resistances influenced by multiple alleles or through different mechanisms, as has been proposed for *P. cerasi*-causing powdery mil-

dew, utilization of a pyramiding approach in sweet cherry breeding offers the potential for longer-term durability against the disease (Olmstead et al., 2001; Baumgartner et al., 2015). Research to identify all available powdery mildew resistance alleles would be useful for informing breeding strategies that exploit different alleles or allelic combinations. Utilization of multiple genetic sources of resistance might offer a long-term, durable disease resistance solution, because "single-gene" resistance can be surmountable, as demonstrated in apple by *P. leucotricha* being capable of overcoming the single resistance source *Pl* for powdery mildew (Caffier and Laurens, 2005) and *Venturia inaequalis* overcoming *Rvi6* for apple scab (Papp et al., 2020). Once specific alleles involved in bacterial canker resistance are found and if alleles conferring reduced infection response to X-disease can be identified, combining the valuable alleles into new cultivars that also carry alleles for superior fruit quality and productivity (Iezzoni et al. 2020) could be effective. Employing suites of resistance alleles for all three pathogens could thus provide new cultivars for the PNW that are able to withstand all three costly diseases.

Conclusion

The future of sweet cherry production lies in being able to economically generate high-quality fruit. While some pathogens such as *P. cerasi* that causes powdery mildew have been well studied, others such as *Ca.* P. pruni that causes X-disease have yet to receive comparable attention. By understanding the physiological mechanisms involved in infection from individual pathogens, treatment options might be discovered that could improve infection outcomes. A longer-term alternative to externally treating an infection each season is genetic resistance to infection in the cultivars grown. Identification of resistance alleles present in sweet cherry germplasm could lead to development of new disease-resistant cultivars that meet industry and consumer needs without posing an ecological risk. Some genetically resistant germplasm of sweet cherry, and influencing alleles, are already known for powdery mildew and bacterial canker, but not X-disease. Ultimately, identifying and exploiting host disease resistance in sweet cherry is expected to powerfully contribute to increased production and profitability via efficient and sustained reduction in infection-incurred loss and environmental impact.

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