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The hop downy mildew pathogen *Pseudoperonospora humuli*

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Abstract

Pseudoperonospora humuli is an obligate biotrophic oomycete that causes downy mildew, one of the most devastating diseases of cultivated hop, *Humulus lupulus*. Downy mildew occurs in all production areas of the crop in the Northern Hemisphere and Argentina. The pathogen overwinters in hop crowns and roots, and causes considerable crop loss. Downy mildew is managed by sanitation practices, planting of resistant cultivars, and fungicide applications. However, the scarcity of sources of host resistance and fungicide resistance in pathogen populations complicates disease management. This review summarizes the current knowledge on the symptoms of the disease, life cycle, virulence factors, and management of hop downy mildew, including various forecasting systems available in the world. Additionally, recent developments in genomics and effector discovery, and the future prospects of using such resources in successful disease management are also discussed.

Taxonomy: Class: Oomycota; Order: Peronosporales; Family: Peronosporaceae; Genus: *Pseudoperonospora*; Species: *Pseudoperonospora humuli*.

Disease symptoms: The disease is characterized by systemically infected chlorotic shoots called "spikes". Leaf symptoms and signs include angular chlorotic lesions and profuse sporulation on the abaxial side of the leaf. Under severe disease pressure, dark brown discolouration or lesions are observed on cones. Infected crowns have brown to black streaks when cut open. Cultivars highly susceptible to crown rot may die at this phase of the disease cycle without producing shoots. However, foliar symptoms may not be present on plants with systemically infected root systems.

Infection process: Pathogen mycelium overwinters in buds and crowns, and emerges on infected shoots in spring. Profuse sporulation occurs on infected tissues and sporangia are released and dispersed by air currents. Under favourable conditions, sporangia germinate and produce biflagellate zoospores that infect healthy tissue, thus perpetuating the infection cycle. Though oospores are produced in infected tissues, their role in the infection cycle is not defined.

Control: Downy mildew on hop is managed by a combination of sanitation practices and timely fungicide applications. Forecasting systems are used to time fungicide applications for successful management of the disease.

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Useful Websites: https://content.ces.ncsu.edu/hop-downy-mildew (North Carolina State University disease factsheet), https://www.canr.msu.edu/resources/michigan-hop-management-guide (Michigan Hop Management Guide), http://uspest.org/risk/models (Oregon State University Integrated Plant Protection Center degree-day model for hop downy mildew), https://www.usahops.org/cabinet/data/Field-Guide.pdf (Field Guide for Integrated Pest Management in Hops).

KEYWORDS
effectors, host resistance, obligate biotroph, oomycete management, oomycetes

1 | INTRODUCTION

Hop (*Humulus lupulus*) is a perennial plant belonging to the family Cannabaceae, used primarily for brewing. The female inflorescence, the strobile, or cone of hop is economically important for imparting the characteristic bitterness, flavour, and aroma in beer (Natsume et al., 2015). Hop cones also contribute to preservation of beer due to their antimicrobial properties (Sakamoto & Konings, 2003).

Downy mildew caused by *Pseudoperonospora humuli* is one of the most devastating diseases of cultivated hop that affects hop production in all regions of cultivation in the Northern Hemisphere and Argentina (Gent et al., 2010). Damage caused by downy mildew can be extensive, causing complete crop loss due to infection of cone-bearing branches and reduced crop quality due to infection of cones (Royle & Kremheller, 1981). Furthermore, downy mildew can overwinter in hop crowns, thereby reducing yield and quality, and in certain cultivars can lead to progressive loss of vigour and plant death in subsequent production seasons (Skotland, 1961).

The disease is primarily managed by timely application of fungicides and cultural practices that reduce inoculum and modify the microclimate of yards, such as spring pruning, removal of excess foliage, and grubbing of heavily diseased plants. Persisting resistance has been reported in the pathogen to multiple classes of fungicides, further complicating management (Gent et al., 2015; Henning et al., 2015). Though host resistance is an ideal strategy for control, known sources of resistance to downy mildew in hop are rare and associated with a narrow genetic base. Resistance to downy mildew is quantitative and attempts have been made to identify quantitative trait loci (QTLs) responsible for downy mildew resistance in hop (Henning et al., 2015). However, breeding for resistance in a perennial crop while maintaining desirable brewing characteristics is a slow process (Woods & Gent, 2016), and difficult because backcrossing is not viable in hop due to severe inbreeding depression (Townsend & Henning, 2005). Identifying and introgressing reliable sources of resistance becomes more complicated because resistance to the crown rot phase and the foliar phase of the disease varies among cultivars, and brewing chemistry traits desired by many brewers are highly complex and tend to be in a genetic background that is highly susceptible to downy mildew (Woods & Gent, 2016).

A thorough understanding of pathogen biology and disease ecology is necessary in order to identify and successfully use new sources of resistance and develop more sustainable disease management approaches. Despite being an economically important pathogen that severely hinders hop production, genomic resources for this pathogen are underdeveloped. The aim of this review is to summarize what is known about hop downy mildew and *P. humuli*, and highlight recently published genomic resources.

2 | TAXONOMY AND MORPHOLOGY of *P. HUMULI*

P. humuli is an obligate oomycete pathogen that was first identified in Japan by Miyabe and Takahashi in 1905 (Miyabe & Takahashi, 1906), and originally named *Peronoplasmopara humuli*. The organism was later renamed as *Pseudoperonospora humuli* by Wilson (Wilson, 1914) due to striking similarities to *Pseudoperonospora celtidis* var. *humuli* from hop plants in the USA. After the initial description of the species in Japan in 1905, downy mildew appeared in hop yards across production areas in the USA and Europe during the period from 1920 to 1930 (Johnson et al., 2009). *P. humuli* belongs to the order Peronosporales, which includes oomycetes causing downy mildews on other plants and the infamous potato late blight pathogen *Phytophthora infestans* (Choi et al., 2005). The genus *Pseudoperonospora* presently comprises four other recognized species, *Pseudoperonospora cubensis* (cucurbit downy mildew), *Pseudoperonospora celtidis* (downy mildew on *Celtis* spp.), *Pseudoperonospora cannabina* (downy mildew on *Cannabis* spp.), and *Pseudoperonospora urticae* (downy mildew on *Urtica* spp.).

P. humuli has dichotomously branched sporangiophores bearing ellipsoid and papillate sporangia. Sporangia are olivaceous brown and have flagellated zoospores (Purayannur, Miles, Gent, et al., 2020). The pathogen produces melanized lemon-shaped sporangia (20–40 × 14–25 μm in diameter) on the abaxial surfaces of leaves. These sporangia are smooth and are borne on a sporangiophore that ranges from 180 to 600 μm in height, with 20 μm diameter, and is 5–7 μm in width (Choi et al., 2005) (Figure 1). *P. humuli* is closely related and similar in morphology to the sister species *P. cubensis* (Mitchell et al., 2011; Salcedo et al., 2020), with overlapping

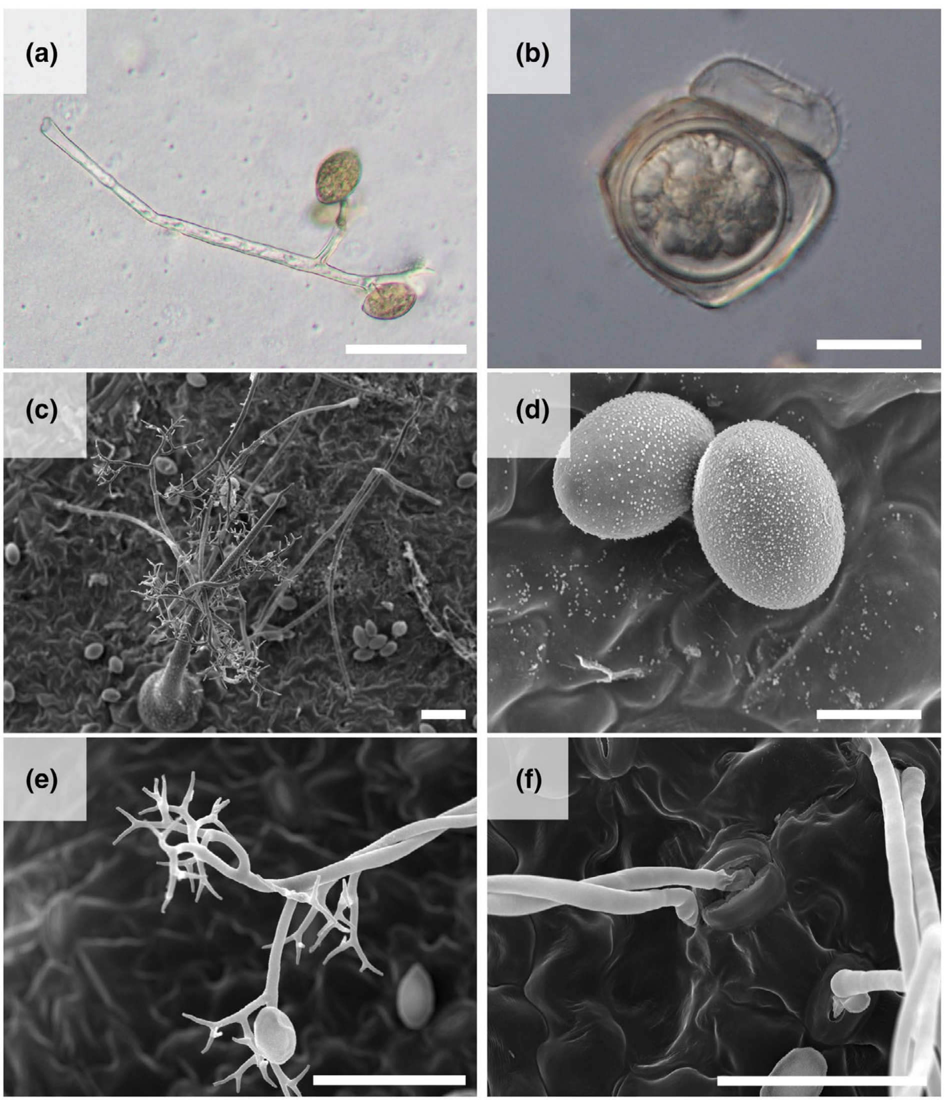

FIGURE 1 Structures of the hop downy mildew pathogen *Pseudoperonospora humuli* visualized with light (a, b) and scanning electron (c–f) microscopy. (a) A sporangiophore and sporangia, (b) an oospore, (c) sporangiophore and sporangia surrounding a hop trichome, (d) sporangia, (e) branch pattern of a sporangiophore following sporangia release, and (f) a sporangiophore emerging from a stomata. Bars are equal to 50 μm in a, c, e, f, and 10 μm in b and d. (Figures c–f courtesy W. Britton)

morphological characters depending on the host substrate and study (Choi et al., 2005). The relatedness of the two species is so pronounced that morphological similarities and internal transcribed spacer (ITS) sequencing led Choi et al. (2005) to reduce *P. humuli* to a taxonomic synonym of *P. cubensis*. However, in closely related downy mildew oomycetes, morphological and ITS-based distinction is inconclusive for resolution of species (Crandall et al., 2018; Rahman et al., 2017; Withers et al., 2016). Other analyses (Göker et al., 2009; Sarris et al., 2009) have included the data set from Choi et al. (2005) with other sequences and found support, albeit weak, for differentiation of *P. cubensis* and *P. humuli*. Although the resolution of the ITS region is limited, both Sarris et al. (2009) and Göker et al. (2009) found that isolates of *P. humuli* originating from the annual species *Humulus scandens* (syn. *Humulus japonicus*) in Asia cluster differentially versus other isolates of *P. humuli* derived from Europe and North America. Mitchell et al. (2011) and Mancino (2013) also found that an isolate of *P. humuli* obtained from *H. scandens* from Asia clustered separately to other isolates of *P. humuli* based on sequencing of other loci.

A species concept based in part on host specialization had been proposed previously for downy mildew pathogens (Thines et al., 2009). To that end, cross-infection assays conducted to differentiate *P. humuli* and *P. cubensis* suggested limited infection potential for *P. humuli* on cucurbits and *P. cubensis* on hop under artificial conditions. This suggests that these organisms are closely related but distinct species (Mitchell et al., 2011; Runge & Thines, 2012; Wallace & Quesada-Ocampo, 2017).

Multigenetic and high-throughput sequencing provided additional evidence for the differentiation of *P. cubensis* and *P. humuli*. A multilocus analysis using the nuclear ITS region, the cytochrome *c* oxidase subunit 2 (*cox2*) gene, and the Ras-related protein (*Ypt1*) showed further separation with high support between *P. humuli* and *P. cubensis* due to the

improved phylogenetic resolution of *cox2* and *Ypt1* (Runge et al., 2011). Additional single nucleotide polymorphisms (SNPs) identified through RNA-Seq and genotyping-by-sequencing (GBS) highlighted the separation of *P. humuli* and *P. cubensis* based on principal component analysis (Summers et al., 2015). Similarly, genome sequencing of *P. humuli* and RNA-Seq in multiple isolates led to the identification of additional high-confidence markers that distinguish *P. humuli* from *P. cubensis* (Rahman et al., 2019; Withers et al., 2016).

3 | SYMPTOMS AND SIGNS

Symptoms of downy mildew manifest on multiple parts of the hop plant from the shoots to the roots. Systemically infected shoots, called spikes due to their resemblance to a wheat spike, are the most distinguishing feature of hop downy mildew (Purayannur, Miles, Gent, et al., 2020). Three types of spikes are usually observed. During spring when conditions are favourable for infection, infected shoots emerge near the base of the plant (Figure 2a). These are called "primary basal spikes". The leaves of the spike are chlorotic and curled downward. Profuse sporulation is present on the abaxial side of the leaves, which serves as inoculum for the spread of the disease. As the disease progresses, the infection spreads to surrounding shoots via sporangia dispersed from the leaves of the infected primary spike, which develop into "secondary spikes" (Figure 2b). Leaves on secondary spikes are also curled downward and chlorotic like those of the primary spikes although the secondary spikes tend to have healthy leaves at the base, which distinguishes them from primary basal spikes (Purayannur, Miles, Gent, et al., 2020; Royle & Kremheller, 1981). An important aspect of hop production is the training of bines (i.e., climbing shoots), which involves wrapping the bines around a string to encourage vertical growth. Trained shoots that become infected cease to grow and fall away from the string and collapse, leading to reduction in yield proportional to the incidence of infection. Infection also may spread through the climbing bines, leading to the emergence of infected branches that are called "lateral spikes" (Royle & Kremheller, 1981) (Figure 2c). Infection of lateral branches causes reduction in yield due to the failure of cones to develop.

On leaves, hop downy mildew appears as angular, vein-delimited lesions that sometimes coalesce during severe infection (Figure 2d). Profuse sporulation is observed on the abaxial surface of the leaf (Figure 2e), sometimes covering the entire leaf surface (Purayannur, Miles, Gent, et al., 2020). Leaf lesions desiccate in warm dry weather, forming brown necrotic tissue. Severe downy mildew can cause defoliation (Royle & Kremheller, 1981).

Downy mildew manifests on cones as a characteristic dark brown discolouration (Figure 2f). Infected cones sometimes appear striped due to uneven discolouration on the bracts and bracteoles (Purayannur, Miles, Gent, et al., 2020). Severe infection can cause malformation and discolouration on the entire cone depending on the timing of infection. Sometimes, sporulation occurs on the underside of the bracts and the bracteoles. However, sporulation on cones is not consistently observed in the field (Gent et al., 2015). Cone infection may result in crop damage through reductions in cone yield and levels of bittering acid and also may lead to rejection of entire crops due to highly conspicuous quality defects.

Root and crown infection appear in the form of brown/black streaks or flecks in the tissue when cut open (Gent et al., 2015) (Figure 2g). The pathogen mycelium perennates in the roots and crown, and can give rise to infected shoots the next season. Some cultivars are highly susceptible to the crown rot phase of the disease and thus die without giving rise to shoots (Coley-Smith, 1964). Unlike other downy mildew diseases caused by pathogens with a systemic phase (Gascuel et al., 2015; Voglmayr et al., 2014), hop plants with systemic infection of the root system may not have any foliar symptoms other than a general reduction in vigour. Susceptibility to the crown rot phase of the disease limits the cultivars that may be produced economically in environments favourable to the disease (Gent et al., 2015).

4 | HOST RANGE, REPRODUCTION, AND POPULATION DIVERSITY

P. humuli may cause limited infection in certain species of the Urticales (Rosales s.l.), which contains the Cannabaceae family. In host range studies with artificial inoculation, *P. humuli* infected *Urtica*, *Cannabis*, and *Celtis* species (Hoerner, 1940; Salmon & Ware, 1928, 1929). However, the infections of these species were accompanied by hypersensitive reactions and sporulation that was sparse compared to *P. humuli* on hop (Hoerner, 1940). Conflicting information is reported on whether *P. humuli* may infect cucurbit hosts. Hoerner (1940) reported that "all attempts to infect available hosts of *Pseudoperonospora cubensis* [with *P. humuli*] … were unsuccessful." Mitchell et al. (2011) found only a single sporangiophore of *P. humuli* when multiple isolates were inoculated at high titres onto cucumber or cantaloupe. In contrast, Runge and Thines (2012) reported that a single isolate of *P. humuli* was able to infect seven of 25 inoculated leaves of cucumber (*Cucumis sativus*), although the density of sporulation was notably less than that of *P. cubensis* inoculated onto the same host. Table 1 of Cohen et al. (2015) reports that a pathotype of *P. cubensis* described in Russia in 2013 can infect hop, but details of this occurrence were not provided. The annual species *H. scandens* may be infected by *P. humuli* at low levels, although this species generally is resistant to downy mildew (Mancino, 2013).

P. humuli is reported to be homothallic (Gent et al., 2017), distinguishing it from the sister species *P. cubensis*, which has been reported to be heterothallic (Cohen & Rubin, 2012). Oospores are spherical and range from 190 to 430 µm in diameter and are sometimes found abundantly in infected tissues in the field (Chee & Klein, 1998; Coley-Smith, 1962; Gent et al., 2017). Oospores are found in the pith tissue of the crown, in buds, and abundantly in cones in most hop-growing areas except arid regions of California, Idaho, and Washington, where oospores are found only on diseased cones (Parker, 2007; Royle & Kremheller, 1981; Skotland & Johnson, 1983).

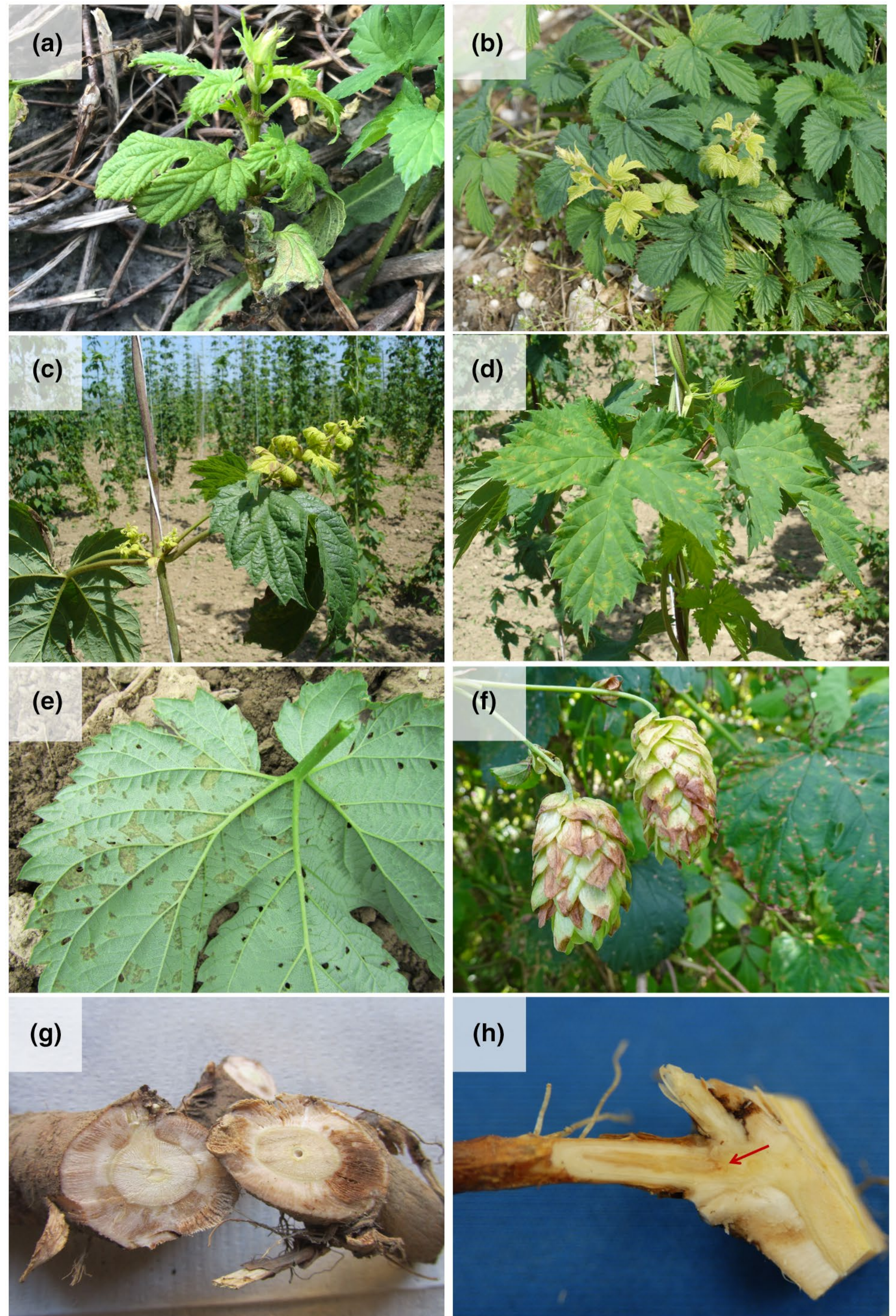

FIGURE 2 Symptoms and signs of hop downy mildew. (a) Primary spikes, (b) secondary spikes, (c) lateral spikes, (d) foliar lesions, (e) sporulation on adaxial leaf surface, (f) cone infection, (g) infected cuttings, and (h) infected bud tissue (red arrow)

Although there are reports of successful germination of oospores (Arens, 1929; Bressman & Nichols, 1933), recent attempts to germinate and infect hop tissue with oospores have failed (Gent et al., 2017). Nonetheless, oospores formed in host tissue appear to be viable (Gent et al., 2017).

Circumstantial evidence that oospores may not be important in the disease cycle is that in both the UK and Washington, USA, hop yards severely affected by downy mildew have been replanted with no disease occurring on the new plants (Coley-Smith, 1962; Skotland, 1961), suggesting that germination of oospores overwintering in soil is not the major source of inoculum the following season. Coley-Smith (1962) found that primary basal spikes did not form from potted plants or healthy cuttings of the bine bases (strap cuttings) inoculated with oospores or with field soil. Primary spikes did form on diseased strap cuttings under the same conditions, but it is uncertain whether oospores produced the spikes.

TABLE 1 Assembly size, number of predicted proteins and RXLRs in *Pseudoperonospora humuli* and other sequenced oomycetes

Species	Assembly size (Mb)	Number of predicted proteins	Number of predicted RXLRs	References
Pseudoperonospora humuli	c.40	18,677	296	Purayannur, Cano, Bowman, et al. (2020); Rahman et al. (2019)
Pseudoperonospora cubensis	c.64	23,522	271, 72	Savory et al. (2012); Purayannur, Cano, Bowman, et al. (2020)
Peronospora tabacina	c.60	c.18,000	c.120	Derevnina et al. (2015)
Peronospora effusa	c.30	13,277	99	Klein et al. (2020)
Hyaloperonospora arabidopsidis	c.81	14,543	134	Baxter et al. (2010)
Plasmopara halstedii	c.75	15,649	274	Sharma et al. (2015)
Plasmopara viticola	c.95	15,960	540	Dussert et al. (2019)

Skotland (1961) performed a search for oospores in the Yakima Valley, Washington, USA, during the period from 1956 to 1960. Oospores were only found once, in a basal spike collected in May 1957. Skotland (1961) concluded that although oospores can be found in the hop-growing areas of Washington, oospores are not commonly produced and probably are not an important source of inoculum in that region.

Due to the obligate nature of *P. humuli* and the inherent difficulties in culturing and maintenance, very little data exist about the population diversity of the pathogen. Chee et al. (2006) compared 40 samples of diseased hop leaves collected from Washington and Oregon, USA, and, using random DNA amplification methods, observed more genotypes of the pathogen in Oregon compared to Washington. The authors attributed the greater genotypic richness to differences in climatic variation between the two regions leading to possible greater occurrence of sexual reproduction in Oregon (Chee et al., 2006). Genetic diversity in *P. humuli* has been reported to be lower than in *P. cubensis* (Wallace & Quesada-Ocampo, 2017), and GBS analysis of a large set of isolates confirmed clonality in populations as expected in a homothallic species (Gent et al., 2019). Unlike the sister species *P. cubensis*, where two distinct host-specialized clades are present (Quesada-Ocampo et al., 2012; Wallace et al., 2020), races or pathotypes have not been reported in *P. humuli* despite several attempts to discern pathogenic variation among isolates (Royle & Kremheller, 1981).

5 | LIFE CYCLE

P. humuli overwinters in dormant hop crowns and gives rise to infected shoots (basal spikes) in favourable conditions (Ware, 1926, 1929). Overwintering mycelium in the systemically infected crown and rootstock spreads into the bud tissue, giving rise to basal spikes. The leaves and stem of the basal spike harbour sporangia that serves as inoculum for the spread of the disease. Sporangia are the most obvious sign of *P. humuli* and they are borne on sporangiophores. High humidity promotes sporangial formation (>80%–90%) and the presence of free moisture releases sporangia (Gent et al., 2010). Each sporangium can discharge five to 15 asexual, ovoid, biflagellate zoospores (10–13 μm in diameter). When these motile zoospores settle on open stomata, they encyst by forming cell walls (Royle & Thomas, 1971a, 1971b, 1973). A germination tube then penetrates the plant cell wall (Johnson et al., 2009) (Figure 3).

Once a host cell wall is penetrated, *P. humuli* hyphae will proliferate within and between host cells. The intercellular mycelium is hyaline and coenocytic, with a diameter of 5.4–7.2 μm. Haustoria are formed within the host cells and allow for the absorption of nutrients. Haustoria are branched, vary in shape, and appear stunted and inflated with clusters of hyphae. Older haustoria often have knob-like structures and callose deposits may surround these structures within the host. Additional sporangiophores emerge from stomata with sporangia on the underside of the leaf (Johnson et al., 2009) (Figure 3).

These initial infections are a secondary source of sporangia for *P. humuli*, which can infect tissues that contain stomata, allowing continuous reproduction during a growing season. Zoospores will continue to infect by entering through open stomata infecting leaves, bud stipules, apical meristems, and cones when temperature and moisture conditions are met. Mild to warm temperatures (15–29 °C) when free moisture is present for at least 1.5–2 hr favours infection (Johnson et al., 2009). Leaf infection can occur at temperatures as low as 5 °C when wetness persists for 24 hr or longer (Royle, 1973). Foliar infections result in localized leaf spots. Systemic shoot infection may occur in a similar fashion but requires a longer period of wetness (3–6 hr) and occurs over a more restricted temperature range (8–23 °C) (Royle, 1970) (Figure 3). Wetness associated with rain appears important for severe infection (Royle, 1973). The most severe infections occur when wetness is coincident with high humidity and relatively warm nights (Gent & Ocamb, 2009; Johnson & Skotland, 1985).

Throughout the season the sexual oospore can form on multiple tissues following antheridial and oogonial plasmogamy and karyogamy (Gent et al., 2017). The role of oospores in the disease cycle is not well understood but oospores are found readily in infected hop tissue and are particularly abundant in diseased cones (Gent et al., 2017; Parker, 2007; Royle & Kremheller, 1981) (Figure 3). However, their role in overwintering is thought to be minimal based on the low

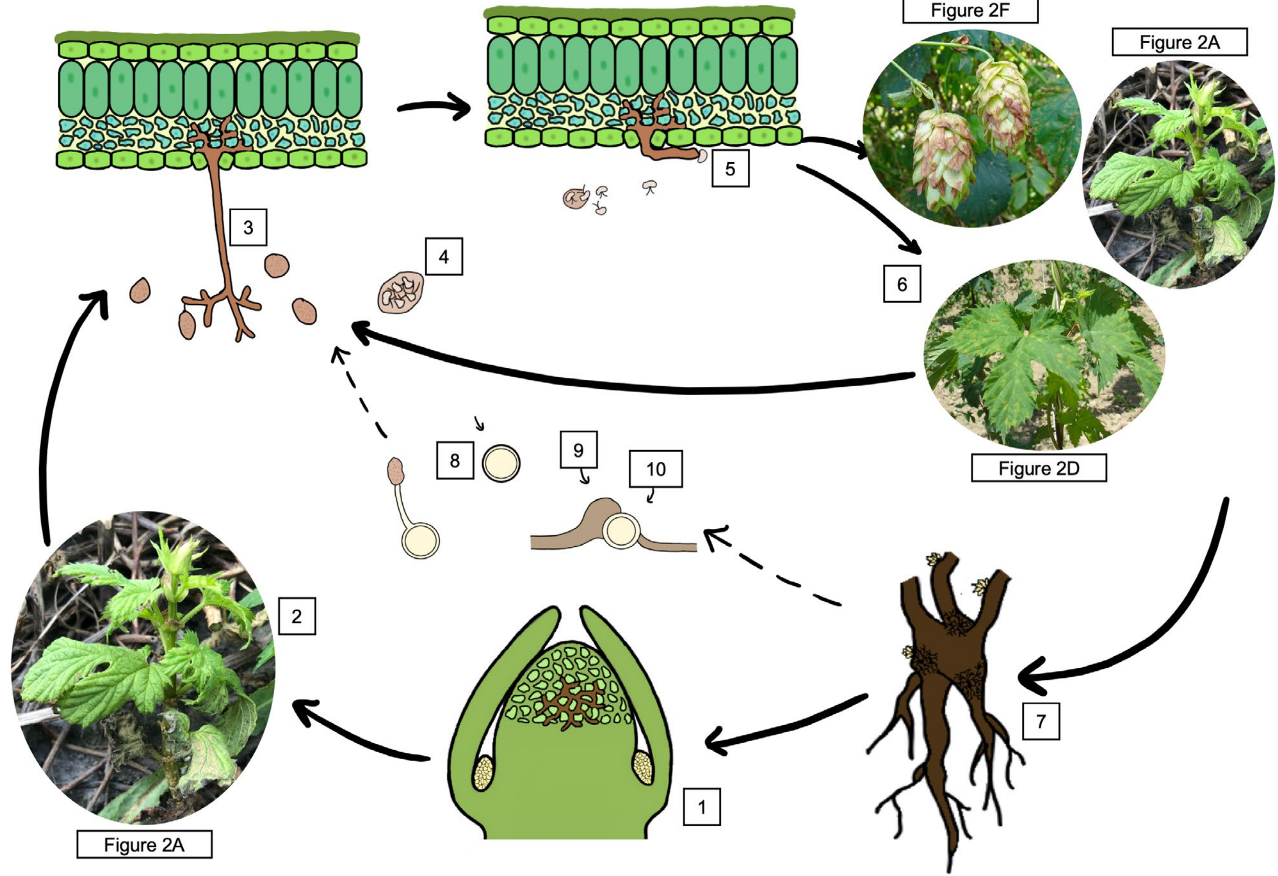

FIGURE 3 Life cycle of *Pseudoperonospora humuli*. (1) Mycelia overwinter in hop crowns and give rise to diseased shoots (2) in spring. Sporangia borne on sporangiophores (3) are present on infected tissue and are released in favourable conditions. Sporangia contain biflagellate zoospores (4) that when released settle on open stomata, penetrate the cell wall through a germ tube, and continue to grow. Sporangiophores appear on the newly infected tissue and the infection cycle continues with zoospores infecting leaves, buds, and cones (6). Infection travels down from aerial parts to hop crowns (7) where the mycelia overwinter. Sexual oospores (8) are formed throughout the infection cycle through fusion of antheridia (9) and oogonia (10), though their role in the infection cycle is not well understood

frequency of germination under controlled conditions and lack of positive evidence for their infectivity (Coley-Smith, 1962; Skotland & Johnson, 1983). In arid climates (e.g., central Washington State, USA) oospores tend to form less frequently (Coley-Smith, 1962; Skotland, 1961). Further investigation is required to evaluate the role of oospore infection in humid continental climates that experience harsh winters.

Infections that occur on the terminal growing point can become systemic, growing down through shoots near the base of the plant toward the crown where the pathogen can persist in the root system. The process of systemic colonization of aerial and belowground tissues is understood only in part. It has been observed that infections occurring at the tip or the base of the shoot can travel down and colonize crown and roots (Coley-Smith, 1962, 1965; Ware, 1926). Although direct infection of rootstock by zoospores has been observed, infections passing from the stem into the rootstock occur more frequently and are probably the major source of rootstock rot (Coley-Smith, 1965). The pathogen overwinters in the roots and crown, and gives rise to infected basal spikes in the following season. Systemic infections contribute to the spread of disease through propagation of infected rhizomes and also allow for the pathogen to survive winter, contributing to disease in subsequent seasons. Diseased rhizomes will often have reddish brown to black flecks and streaks present within the tissue (Johnson et al., 2009). The pathogen can be found in several portions of the root, including the pith, cortex, and epidermis (Skotland, 1961). Ultimately, these infections can lead to plant death in susceptible hop cultivars (Coley-Smith, 1962; Royle & Kremheller, 1981; Woods & Gent, 2016) (Figure 3).

6 | RESISTANCE IN HOP

Growing resistant or tolerant cultivars is a cost-effective solution for management of downy mildew in hop. However, sources of resistance to downy mildew among hop cultivars are scarce and associated with a narrow genetic base. High levels of resistance can be found in cultivars developed in Europe, such as Magnum, Challenger, and Orion (Parker, 2007; Woods & Gent, 2016). Partial resistance is more common in commercial cultivars, such as Newport (Henning et al., 2004) and Teamaker (Henning et al., 2008). In general, cultivars

presently in most demand by craft brewers are relatively susceptible to the disease (Woods & Gent, 2016). Fully resistant cultivars are not commercially grown in large acreage due to brewing quality attributes or other agronomic limitations but are used as breeding stock to develop new cultivars (Parker, 2007). It has been a priority for hop breeders to develop germplasm to aid in the generation of new resistant cultivars. Nonetheless, progress in breeding for downy mildew resistance has been incremental and slow. Cultivars and other germplasm with the highest levels of resistance to downy mildew can be traced back to germplasm developed in Germany by Zattler (Henning, 2006). Recently, a male hop with relatively high resistance to downy mildew was made available for breeding programmes (Henning et al., 2018). The genetic background of this male is believed to be distinct from the germplasm developed by Zattler and progeny thereof.

Attempts at identifying the mode of inheritance indicate that resistance to downy mildew in hop is quantitatively controlled (Henning et al., 2015; Parker, 2007). Several attempts have been made to identify markers with association to downy mildew resistance (Henning et al., 2015, 2016; Parker, 2007). Parker (2007) characterized 43 amplified fragment length polymorphism (AFLP) markers using 99 hop accessions phenotyped based on percentage of leaf infection in a single environment. Recent developments in sequencing techniques have enabled linkage mapping and QTL analysis (Henning et al., 2015), and genome-wide association studies (GWAS) (Henning et al., 2016) in a biparental mapping population of 125 genotypes phenotyped in different environments. Henning et al. (2015) identified different QTLs for downy mildew resistance based on field data obtained from Oregon and Washington States in the USA, and greenhouse data. Considering the variation between environments observed and taking into account the different criteria for phenotyping in a greenhouse (leaf area infected) and field data (percentage infected shoots), Henning et al. (2015) opined that screening under both conditions would ensure greater selection success. Genotyping-by-sequencing in the same biparental population identified SNP markers significantly associated with downy mildew resistance based on the same environments as the previous study. Some of the identified markers were tested using high resolution melt curve analysis and four SNPs with significant association were identified. These markers need to be validated in different genotypes (Henning et al., 2015) due to the highly complex genome of hop (Easterling et al., 2018; Zhang et al., 2017). Preformed metabolites associated with the phenylpropanoid pathway in hop also have been correlated with resistance to the foliar phase of downy mildew (Feiner et al., 2021).

Henning et al. (2004) classified various hop accessions in North America into three distinct genetic diversity pools based on yield and chemistry, although disease resistance was not a criterion. Woods and Gent (2016) assessed the disease susceptibility of 110 accessions under field conditions in Oregon, USA, 79 of which were those included in the study conducted by Henning et al. (2004). Cultivars originating from Europe were found to exhibit more vigour (expressed as the number of shoots produced) and resistance to downy mildew than those from USA, Japan, and Australia/New Zealand, although the authors argued that this could partially be attributed to vigorous selection of downy mildew resistance in breeding programmes in Europe (Woods & Gent, 2016). Similar results were obtained by Dolinar and Kralj (1995) in Slovenia with the assessment of more than 100 accessions under field conditions and by a leaf infection assay. They found higher susceptibility in a group of genotypes that originated from South Africa, Australia/New Zealand, and the USA, while European germplasm showed higher resistance. They also identified several highly resistant genotypes that originated from Japan and China (Dolinar & Kralj, 1995).

Disease assessment can get complicated because of the differences in susceptibility of cultivars to the crown rot phase and the foliar phase of the disease. While some cultivars are more susceptible to the former, the others are to the latter (Woods & Gent, 2016). Cultivars highly susceptible to the crown rot phase may die before producing shoots (with or without symptoms), making field assessments of disease susceptibility to both phases difficult (Woods & Gent, 2016).

In the absence of disease, North American germplasm tends to produce higher yields than the European germplasm and possesses desired aroma and flavour qualities (Henning et al., 2004), complicating breeding approaches. No correlation was observed between susceptibility to downy mildew in the accessions tested by Woods and Gent (2016) to the traits assessed by Henning et al. (2004), except for a strong negative correlation between cohumulone levels in the shoots and the number of shoots produced per plant. High levels of cohumulone are characteristic of hop germplasm derived from the North American gene pool (Henning et al., 1997).

7 | GENOME RESOURCES AND VIRULENCE FACTORS

A draft genome assembly of the *P. humuli* isolate OR502AA collected from the hop cultivar Centennial was recently published (Rahman et al., 2019). The size of the assembled genome was estimated to be 47.2 Mb using the k-mer profiles of the DNAseq reads with GenomeScope although the genome size had been earlier estimated to be c.80 Mb using Feulgen absorbance cytophotometry by Voglmayr and Greilhuber (1998) (Table 1). Rahman et al. (2019) argue that their estimation is probably more precise because it is closer to the final assembled genome size of c.40 Mb but a better assembly using long-read sequencing might resolve the differences in the future. The genome assembly of *P. humuli* has 18,677 predicted coding genes of which 53% have evidence of expression based on the transcriptomes of eight different isolates (Rahman et al., 2019). The mitochondrial genome of *P. humuli* is a circular molecule of 39 kb (Rahman et al., 2019), which is similar in size to some other analysed downy mildew mitochondrial genomes such as *P. cubensis* (38.5 kb) (Savory et al., 2012) and *Peronospora tabacina* (43 kb) (Derevnina et al., 2015).

Plant pathogens secrete effectors that modulate host metabolic processes to facilitate infection. Effectors can be classified into

apoplastic and cytoplasmic effectors based on host cell localization (Schornack et al., 2009). Oomycete pathogens have two well-characterized modular cytoplasmic effector classes, the RXLRs and the CRNs (Bozkurt et al., 2012). Both classes of proteins possess conserved amino acid motifs: the RXLR and the EER motifs in the RXLR class and the LXLFLAK and HVLVVVP motifs in the CRNs. Varying numbers of RXLR and CRN effectors have been identified in downy mildew pathogens (Baxter et al., 2010; Derevnina et al., 2015; Dussert et al., 2019; Sharma et al., 2015). Rahman et al. (2019) identified 189 RXLRs and 49 CRNs in the *P. humuli* isolate OR502AA in an initial analysis.

Recently, a comprehensive scan of the *P. humuli* genome was conducted to identify putative apoplastic and cytoplasmic effectors (Purayannur, Cano, Bowman, et al., 2020). The *P. humuli* secretome consisted of 1,250 proteins of which 321 were putative apoplastic effectors. Apoplastic effectors identified in *P. humuli* consisted of known classes such as carbohydrate-active enzymes (CAZymes), glucanase inhibitors, protease inhibitors, necrosis and ethylene-inducing peptide 1-like proteins (NLPs), and sperm coat proteins (SCPs). No CRNs were identified in the *P. humuli* secretome although there were 53 proteins containing the characteristic LXLFLAK and/or HVLVVVP motifs without predicted signal peptides (Purayannur, Cano, Bowman, et al., 2020). There were 296 RXLR-like proteins in *P. humuli*, which is a higher number than that of some other downy mildew pathogens (Table 1) (Baxter et al., 2010; Derevnina et al., 2015; Sharma et al., 2015). The number is closer to the 271 predicted RXLRs in the sister species *P. cubensis* (Savory et al., 2012), even though the analysis conducted by Purayannur, Cano, Bowman, et al. (2020) revealed only 72 predicted RXLRs in the Savory et al. (2012) *P. cubensis* genome, a discrepancy probably due to the RXLR prediction pipeline in the two studies. While Savory et al. (2012) included proteins with different amino acids in the R1 position of the RXLR motif, Purayannur et al. (2020) included such noncanonical proteins only when a downstream EER motif was present. Additionally, Purayannur et al. (2020) used a modified version of the *P. cubensis* proteome after filtering for possible bacterial contaminant proteins. This raises an important point that differences in protein prediction pipelines can cause drastic changes in the numbers of RXLRs in a proteome. Some RXLRs possess an additional motif in the C-terminus involving repeats of the amino acids W, Y, and L (Haas et al., 2009; Jiang et al., 2008). Recent reports emphasize the importance of WY domain-containing effectors that lack a canonical RXLR motif in downy mildew pathogens (Derevnina et al., 2015; Wood et al., 2020). In line with this, there were 74 such effectors in the secretome of *P. humuli* (Purayannur, Cano, Bowman, et al., 2020).

Transcriptome analysis in 12 isolates of *P. humuli* showed evidence for 171 apoplastic and 296 RXLRs in all the isolates, suggesting that these are conserved. Time-course RNA-Seq analysis with infected foliar tissue showed temporal elevation in the expression of some effectors. Overall, there were 75 core effectors in *P. humuli* that showed conserved transcript evidence in all isolates and elevated expression during infection (Purayannur, Cano, Bowman, et al., 2020).

In resistant plants, effectors are recognized by R proteins encoded by *R* genes, leading to a visually apparent localized cell-death response, which can be used to screen for and select potential sources of resistance in the absence of pathogen and associated environmental variance. This approach is called effector-assisted breeding (Vleeshouwers & Oliver, 2014). Core effectors of *P. humuli* can be now used to identify new sources of resistance in hop germplasm.

8 | DISEASE MANAGEMENT

The fundamental approaches to management hop downy mildew surprisingly have changed little over the past century. Successful disease management integrates sanitation measures that reduce initial inoculum and modify the environment to be less favourable to disease, selection of less susceptible cultivars when possible, and timely application of fungicides (Gent et al., 2015; Royle & Kremheller, 1981). Though resistance varies quantitatively among cultivars, no cultivars that are widely planted are completely resistant to the disease. Furthermore, the required level of resistance depends on the environmental conditions of the geographical region in which the crop is grown (Gent et al., 2015; Henning et al., 2015). For example, cultivars that are tolerant to the crown rot phase may develop the disease only occasionally when grown in semi-arid environments but require repeated fungicide applications when grown in a maritime climate to suppress the foliar phase of the disease. In regions with humid summers such as the midwestern and eastern USA, downy mildew management tends to drive all major management decisions and repeated drench and foliar fungicides are required (Higgins et al., 2020).

Cultural practices are important to prevent the introduction and onset of the disease. Due to the systemic nature of *P. humuli* on hop, the pathogen tends to persist in a given hop yard and result in polyetic epidemics (Coley-Smith, 1962; Gent et al., 2010; Johnson & Anliker, 1985). There is some evidence of a founder effect with *P. humuli*, whereby the initial population of the pathogen introduced into a hop yard persists over time (Gent et al., 2019). Thus, selection of disease-free planting material during the establishment of a new yard delays disease onset and chronic infections (Skotland & Johnson, 1983). The European and Mediterranean Plant Protection Organization (EPPO) has standards for the production of certified pathogen-tested plant materials for hop (EPPO, 2009). These standards recommend visual assessment for downy mildew and specify visual symptoms on no more than 1% of plants in certified stock. We are unaware of any of the available molecular diagnostics currently in use in certification schemes, although the potential benefit seems clear. Furthermore, EPPO standards indicate an “appropriate and effective” plant protection programme should be followed during propagation. In practice, attempts are made by commercial propagators to keep disease levels as low as feasible and physically separate propagation facilities from commercial production. Removal of heavily diseased plants is also recommended (Coley-Smith, 1964).

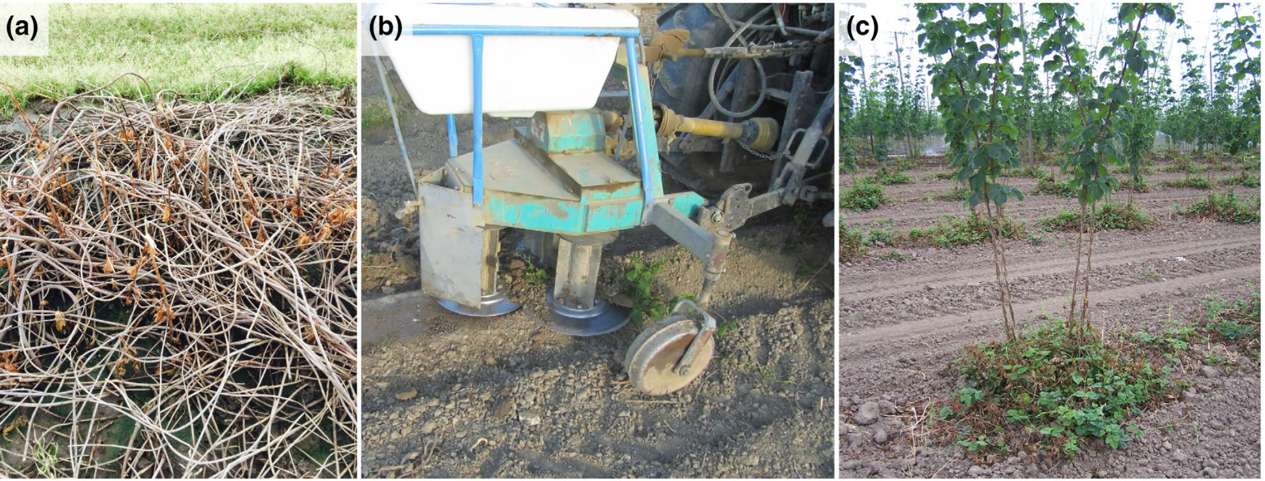

FIGURE 4 Cultural management practices for hop downy mildew. (a) Spring pruning by means of desiccant herbicides, (b) mechanical pruning by a double-disk mechanical pruner, and (c) stripping of lower leaves

Sanitation measures that eliminate primary inoculum are critical. This involves planting disease-free rhizomes or potted plants, and prevention of crown infection by means of chemical protection of crowns and young shoots (Coley-Smith, 1965). Thorough removal of surface crown buds and the associated new wood by means of chemical (Figure 4a) or mechanical (Figure 4b) means substantially reduces disease severity (Gent et al., 2010, 2012). There was a strong association between disease severity and the amount of green tissue remaining after spring pruning in studies in Oregon, USA (Gent et al., 2012). Disease is suppressed by delaying pruning as long as possible, although delayed pruning may reduce yield depending on cultivar and environmental conditions (Gent et al., 2012; Rybáček, 1991). Why delayed pruning suppresses disease is not known with certainty, but probably reflects reducing the dose of primary inoculum, moderation of later canopy development, and an overall shortening of epidemic duration.

As growth resumes following spring pruning, canopy management becomes a key aspect of disease mitigation. In Europe, and to a lesser degree in the western USA, superfluous basal foliage is removed regularly to eliminate inoculum and control the microclimate of hop yards (Gent et al., 2016; Romanko, 1964; Royle & Kremheller, 1981). Basal foliage may be stripped by hand, mechanically, and/or using chemical desiccant herbicides or solutions of nitrogen fertilizers (Figure 4c). Cover crops and weeds between hop rows also are actively managed to promote airflow and reduce humidity.

Aside from the selection of disease-free planting material and sanitation practices, timely application of fungicides is required for the management of hop downy mildew in most production regions. Multiple fungicides are efficacious, including those classified by the Fungicide Resistance Action Committee as groups 4, 11, 21, 27, 40, 43, 45, 49, M1, and P7 (formerly group 33). Activity of fungicides in controlling hop downy mildew is mostly preventative, with limited efficacy after infection (Gent et al., 2015). Hence it is imperative to forecast disease and time fungicide applications appropriately. Resistance to group 4 (mefenoxam) and group P7 (phosphonate) fungicides is known in *P. humuli* (Gent et al., 2020; Hellwig et al., 1991; Klein, 1994; Nelson et al., 2004). To date, resistance has not been found to group 40 (mandipropamid and dimethomorph), which is in contrast to *P. cubensis*, which contains well-characterized SNPs that are associated with resistance (Blum et al., 2012; Higgins et al., 2020).

The timing of fungicide applications is important for effective disease control. Early season application of protectant fungicides is critical for preventing systemic infections of crowns (Coley-Smith, 1965) and minimizing secondary spread of disease in heavily diseased yards (Coley-Smith, 1966). Highly systemic fungicides such as fosetl-Al and mefenoxam are preferentially used early in the season to suppress development of basal spikes; contact fungicides tend to be used later in the season to suppress secondary infections. Various forecasting systems have been established to assist with timing application of fungicides (Dolinar, 1985; Gent et al., 2010; Johnson & Coil, 1989; Johnson et al., 1983; Kremheller, 1979; Royle, 1979). Commercial forecasting systems are available (Adcon Telemetry GmbH) and have been implemented in the UK and Slovenia to predict downy mildew risk based on relative humidity, rain, and temperature (Gent et al., 2010; Royle, 1979). In the Czech Republic, a hop downy mildew index is calculated and issued based on similar meteorological data (Pejmal et al., 1978). In addition to meteorological data, disease incidence on leaves, flowers, and cones are evaluated at 15-day intervals from June until harvest and used for downy mildew prognosis in the Czech Republic (Vostrel et al., 2009). Forecasting systems in Continental Europe use similar risk factors, but also use sporangial density as inputs (Dolinar & Žolnir, 1994; Kremheller & Diercks, 1983). In Germany and Slovenia, the concentrations of airborne sporangia are monitored using volumetric spore traps that are located in a subset of representative hop yards of susceptible and more tolerant varieties, as well as in areas with different climatic conditions. It is assumed that airborne sporangia concentrations are relatively uniform, especially later in the season, and that spore trap data are generalizable to similar varieties in the

region. Disease warnings are based on sporangia thresholds that are defined for pre- and post-flowering periods. In Washington State, the forecasting system of Johnson and Coil (1989) used disease incidence in a hop yard as a predictor of airborne sporangial inoculum potential. An important prerequisite condition for forecasting of secondary infections is control of primary infection, which reduces the potential for diseased shoots to emerge from systemically infected rootstocks.

P. humuli is presumed to be highly dispersible by wind based on dispersal characteristics of the sister species *P. cubensis* (Jaing et al., 2020; Ojiambo et al., 2015). However, models for prediction of long-distance dispersal have not been developed for *P. humuli*. PCR-based assays have been used to detect and quantify sporangia in the air in hop yards (Crandall et al., 2021; Gent et al., 2009; Rahman et al., 2019; Summers et al., 2015). These marker systems are based on unique gene regions (Crandall et al., 2020; Rahman et al., 2019), or SNPs in genes or spacer regions (Gent et al., 2009; Summers et al., 2015) similar to *P. cubensis* (Rahman et al., 2020). Degree-day models have been developed to predict the emergence of basal spikes in the Pacific Northwest of the USA (Gent et al., 2010; Johnson, 1991). Such models aid in determining when disease monitoring should begin or time the first fungicide application of the season, thereby reducing the spread of early season downy mildew. Different disease forecasting systems, such as those described previously, are used later in the season. Direct detection of sporangia in the air of hop yards also has been used to time the first fungicide application (Gent et al., 2009).

9 | FUTURE PROSPECTS

Downy mildew remains one of the most serious diseases that threatens commercial hop production. Integrated management strategies involving fungicide applications and sanitation practices usually can control hop downy mildew. However, the costs of these efforts, increasing scrutiny of pesticide use, and development of fungicide resistance complicate disease management.

Though planting resistant cultivars is a desirable strategy to control hop downy mildew, sources of resistance are rare and complete resistance has not been identified. Furthermore, differences in susceptibility of cultivars in different geographical locations to the crown rot and foliar phases of the disease complicates selection of tolerant cultivars (Woods & Gent, 2016). Detailed disease assessment of foliar and crown rot disease symptoms in cultivars could address this problem. More broadly, the host, pathogen, and environmental factors that permit or restrict systemic infections are little studied. A deeper understanding of systemic infections would be informative for management in this pathosystem and potentially other downy mildews, as systemic infection is not uncommon among these pathogens (Voglmayr et al., 2014). Leveraging knowledge of pathogen effectors for effector-assisted breeding to screen natural sources of resistance would help in efficiently identifying complete sources of resistance to downy mildew. Core effectors that are present and expressed during infection in multiple isolates are ideal candidates for effector-assisted breeding. In *P. humuli*, core effectors have been identified through RNA-Seq analysis (Purayannur, Cano, Bowman, et al., 2020) in foliar tissue and may be used to screen available and new hop germplasm for resistance. Additionally, comparative RNA-Seq between infected crown and foliar tissue may help in identifying differentially expressed virulence factors in these two phases of the disease. In addition to identifying sources of resistance, functional studies need to be performed on identified *P. humuli* effectors to identify potential targets in the host that can then be used for breeding. Combining disease resistance with the suite of desirable horticultural characteristics and brewing quality attributes required by growers and brewers remains a critical but difficult area for future research and development.

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DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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