

REGISTRATION

Germplasm

Registration of seven powdery mildew-resistant wild hop germplasm lines

Joshua S. Havill¹  | Michele S. Wiseman² | John A. Henning³ | David H. Gent³ | Gary J. Muehlbauer¹

¹Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul, MN 55108, USA

²Dep. of Botany and Plant Pathology, Oregon State Univ., Corvallis, OR 97331, USA

³USDA-ARS, Forage Seed and Cereal Research Unit, Corvallis, OR 97331, USA

Correspondence

Gary J. Muehlbauer, Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, St. Paul, MN 55108, USA.

Email: muehl003@umn.edu

Assigned to Associate Editor Arron Carter.

Registration by CSSA.

Funding information

Minnesota Department of Agriculture, Grant/Award Numbers: 138705, 3000027025; Hopsteiner, S. S. Steiner, Inc. JSH was also supported by fellowships from the Plant and Microbial Biology graduate program at the University of Minnesota

Abstract

Cultivation of common hop (*Humulus lupulus* L.) has increased dramatically during the past decade, with the primary growth occurring in the Pacific Northwest of the United States. A major limitation to hop production is hop powdery mildew [caused by *Podosphaera macularis* (Wallr.) Braun & Takam]. To date, a comprehensive evaluation of wild hop germplasm resources for resistance to hop powdery mildew has yet to be conducted. Herein, we report the first extensive powdery mildew resistance screening of 244 wild *H. lupulus* populations (6,732 individuals) collected from North America and Eurasia. We used an elimination screening approach by sequentially inoculating four *P. macularis* isolates containing virulence on all previously described *R* genes. We detected the presence of powdery mildew resistance to isolates representing extant pathogenic diversity in seven germplasms (UMN-PMR-002 Male [Reg. no. GP-38, PI 699936], UMN-PMR-005 [Reg. no. GP-39, PI 699937], UMN-PMR-006 Female [GP-40, PI 699938], UMN-PMR-007 Male [Reg. no. GP-41, PI 699939], UMN-PMR-008 [Reg. no. GP-042, PI 699940], UMN-PMR-010 Male [Reg. no. GP-43, OU 699941], UMN-PMR-013 Female [Reg. no. GP-44, PI 699942]). Based upon genotypic profiling, the seven germplasms were identified in *H. lupulus* var. *lupulus* originating from Eurasia. These accessions could be used to introgress broad-spectrum powdery mildew resistance in hop breeding programs worldwide.

1 | INTRODUCTION

Common hop (*Humulus lupulus* L., Cannabaceae) is a diploid, dioecious, wind-pollinated, climbing, perennial plant native to temperate regions of the Northern Hemisphere (Moir, 2000; Neve, 1991). The female inflorescence (or cone)

contain resins and essential oils that impart bitterness, flavor, and aromatic properties to beer (Neve, 1991). Initial commercial production used landrace varieties, or wild *H. lupulus* genotypes, that were selected based on clonal evaluation for brewing attributes and overall agronomic performance (Brooks, 1962; Darby, 2007). Current cultivars were derived from crossing only a few founder genotypes resulting in a narrow genetic base in cultivated hop, which limits the potential for genetic improvement and increases the risk of multiple cultivars succumbing to similar strains of a pathogen (Darby,

Abbreviations: DPI, days postinoculation; RT-qPCR, reverse transcriptase quantitative polymerase chain reaction; SNP, single nucleotide polymorphism.

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2007; Gent et al., 2017; Neve, 1991; Wolfenbarger, Massie, et al., 2016).

Hop powdery mildew [caused by the obligate plant parasite *Podosphaera macularis* (Wallr.) Braun & Takam] causes significant economic losses in commercial hop production (Gent et al., 2008; Gent et al., 2016; Ocamb et al., 1999; Turechek et al., 2001). *Podosphaera macularis* has recently invaded numerous production areas (Allan-Perkins et al., 2019; Marks et al., 2018; Wolfenbarger, Quesada-Ocampo, et al., 2016) and has been identified as a contributing factor for the collapse of the northeastern United States hop industry in the early 20th century (Blodgett, 1913, 1915). The increased prevalence of highly-virulent strains of hop powdery mildew fungus have been documented in established production regions such as the U.S. Pacific Northwest and Germany and resulted in reduced yield and quality (Gent et al., 2017; Seigner et al., 2006; Wolfenbarger, Massie, et al., 2016). Fungicides are widely used and effective but add cost, result in environmental problems, and may lead to fungicide-resistant isolates (Claassen et al., 2022; Wyenandt et al., 2010). Thus, there are compelling reasons to identify and deploy novel sources of hop powdery mildew resistance.

Several putative *R* genes have been identified in *H. lupulus* that confer resistance to *P. macularis* (termed *RB*, *R1*–*R6*), which appear to follow the gene-for-gene hypothesis. Their effectiveness is dependent upon the presence of pathogen strains containing the corresponding avirulence genes (Darby, 2001; Seigner et al., 2002; Wolfenbarger, Massie, et al., 2016). Some previously effective *R* genes are reported as being ineffective to *P. macularis* in major production regions of the United States, Germany, and the United Kingdom (Gent et al., 2017; Seigner et al., 2006). Therefore, novel sources in combination with extant effective sources of genetic resistance to *P. macularis* are required to reduce damage caused by the disease (Gent et al., 2018; Seigner et al., 2006).

Wild *H. lupulus* may be a rich resource of genetic diversity for disease resistance or other traits (Gent et al., 2018; Patzak et al., 2010; Smith, 2005), but the underlying genetic basis of these traits remains unclear in this germplasm. *Humulus lupulus* can be subdivided into five botanical varieties, which include *H. lupulus* var. *cordifolius* (Miq.) Maxim., *H. lupulus* var. *lupuloides* E. Small, *H. lupulus* var. *lupulus*, *H. lupulus* var. *neomexicanus* A. Nelson & Cockerell, and *H. lupulus* var. *pubescens* E. Small (Small, 1978, 1980). There is also limited information regarding pathogen variation in eastern North America (Weldon et al., 2021; Wolfenbarger, Massie, et al., 2016; Wolfenbarger, Quesada-Ocampo, et al., 2016), so *R* genes deployed in commercial hop cultivars grown in nascent hop production areas may already be overcome as a result (Gent et al., 2017, 2018).

The overall goal of this work was to identify putative novel sources of genetic resistance in a collection of wild germplasm resources by using an elimination screening

Core Ideas

- New hop powdery mildew resistant sources were identified from wild Eurasian hops.
- Rootstocks of powdery mildew resistant sources were deposited in the National Plant Germplasm System.
- Biological sex of powdery mildew resistant germplasm was determined to aid in future breeding and genetic studies.

approach with different pathogenic races. We also sought to clarify the genetic origin of a set of resistant genotypes and deposit pathogen-screened germplasm containing broad-spectrum resistance to hop powdery mildew into the USDA-ARS National Clonal Germplasm Repository for use by the larger research community. We detected the presence of powdery mildew resistance to isolates representing extant pathogenic diversity in seven germplasm lines (UMN-PMR-002 Male [Reg. no. GP-38, PI 699936], UMN-PMR-005 [GP-39, PI 699937], UMN-PMR-006 Female [Reg. no. GP-40, PI 699938], UMN-PMR-007 Male [Reg. no. GP-41, PI 699939], UMN-PMR-008 [Reg. no. GP-42, PI 699940], UMN-PMR-010 Male [Reg. no. GP-43, PI 699941], UMN-PMR-013 Female [Reg. no. GP-44, PI 699942]). Based upon genotypic profiling, the seven germplasm lines were identified in *H. lupulus* var. *lupulus* originating from Eurasia.

2 | METHODS

2.1 | Identification of virulence and pathogenicity of a Minnesota *P. macularis* isolate

We collected *P. macularis* from an invasive population of Japanese hop (*H. japonicus* Sieb. & Zucc.) growing in southeastern Minnesota. Detached leaves of the powdery mildew susceptible hop cultivar ‘Pacific Gem’ (PI 617282) were rub-inoculated with powdery mildew-affected Japanese hop leaves. Fourteen days postinoculation (DPI), singular colonies were purified by re-inoculating onto detached leaves of the same cultivar by removing an isolated conidial chain and transferring to a new detached leaf. Subsequently, at 14 DPI, a purified culture was selected at random and inoculated onto each of three detached leaves of a differential set of hop cultivars containing previously described *R* genes (Gent et al., 2017; Wolfenbarger, Massie, et al., 2016).

This differential set contains the cultivars ‘Northern Brewer’ (*R0*, universal susceptible), ‘Yeoman’ (*RB*), ‘Zenith’ (*R1*), ‘Target’ (*R2*), ‘Challenger’ (*R3*), ‘Serebrianka’ (*R4*),

‘Early Choice’ (R5), and ‘Nugget’ (R6) (Gent et al., 2017). Virulence was assessed 14 DPI as the macroscopic presence of powdery mildew. Three additional leaves of Northern Brewer were left noninoculated, with each isolate tested as a negative control.

2.2 | Plant material and isolates used for resistance screening

Seed germplasm, softwood cuttings, and rhizomes were sampled from the USDA National Clonal Germplasm Repository (USDA-NCGR) or were privately collected from wild common hop populations in the United States, France, Russia, or Japan. In total, there were 6,732 plants screened. Based upon geographic sampling, these plants were thought to represent all five described intraspecific botanical varieties of *H. lupulus*, which include *H. lupulus* var. *cordifolius* (Miq.) Maxim., *H. lupulus* var. *lupuloides* E. Small, *H. lupulus* var. *lupulus*, *H. lupulus* var. *neomexicanus* A. Nelson & Cockerell, and *H. lupulus* var. *pubescens* E. Small (Small, 1980). Seed for individual populations was sown in flats containing moistened Sunshine Mix #8 (Sun Gro Horticulture), lightly covered with coarse vermiculite, and placed at 4 °C for a period of 8 wk for stratification. Germinated seedlings were transplanted into small pots (0.135 L) using a 4:1 ratio of Sunshine Mix #8 and Turface MVP (Profile Products LLC). Collected rhizomes were grown in 3.8-L pots using the same soil mix as transplanted seedlings. Plants produced from softwood cuttings of rhizomes were first propagated using single-node cuttings in HortiCubes (Oasis Grower Solutions), transplanted 10–14 d later, and treated similarly to the seedling plants. All plants were grown and maintained in a greenhouse free from powdery mildew by vaporizing sulfur daily. Plants were irrigated every other day with Peter’s Professional 20–10–20 N–P–K fertilizer (ICL Specialty Fertilizers). Plants were grown to the three-node stage, and prior to inoculation, sprayed with 70% ethanol to remove residual sulfur and washed with tap water for 30 s. Pathogen isolates of *P. macularis* were maintained on either detached leaves following the methods of Wolfenbarger, Massie, et al. (2016) or on whole plants of the susceptible cultivars Northern Brewer or Pacific Gem and were transferred every 2 wk. Inoculum was produced by washing conidia from affected leaves of diseased plants with sterile Milli-Q water (Millipore-Sigma) amended with Tween 20 (0.05%) (Fischer Scientific), strained through MiraCloth (Millipore-Sigma), and adjusted to 5.0×10^4 conidia mL⁻¹ with the aid of a hemacytometer. Plants were inoculated until runoff with an airbrush sprayer. Following inoculation, the plants were allowed to air dry and then placed into a growth chamber set at 18 °C with a 16-h photoperiod. Disease was rated on inoculated plants at 14 DPI using a five-step ordinal scale (0–4) described as follows: 0 = completely resistant; 1 = one

or a few colonies, limited sporulation (if any) or flecking (hypersensitive response); 2 = few discrete colonies on multiple leaves; 3 = coalescing colonies on entire leaves, usually restricted to inoculated leaves; and 4 = all leaves covered in coalescing colonies.

Following the methods of Gent et al. (2018), random subsets of 576 plants were screened during each time point along with a replicated set of control plants including Northern Brewer, Yeoman, Zenith, Target, Challenger, Serebrianka, Early Choice, and Nugget to ensure infectivity of inoculum and to detect possible changes in virulence patterns (Gent et al., 2017; Seigner et al. 2006). Each group of plants were inoculated with a single isolate of *P. macularis* originating from Minnesota (MN-001, race VB, V3, V5), until the entire collection was screened once. All individuals receiving a score of 0 were reinoculated to confirm the initial findings and limit the potential of escapes (false negatives). Individuals receiving a different disease score in the second screening were then designated the highest disease score between either experiment.

After screening all genotypes with one pathotype, individuals with a score of 0 were selected for further screening with a second pathogen race (VB, V3, V4, V5, V6, isolate HPM-666) (Wolfenbarger, Massie, et al., 2016). For these experiments, all resistant individuals were replicated by producing softwood cuttings, and sets of three plants were inoculated in a similar manner alongside control plants of the hop cultivars Nugget and Northern Brewer. Plants were rated 14 DPI using the same 0–4 scale. Inoculations were performed a second time on an additional set of softwood cuttings. A third round of inoculations on softwood cuttings was conducted using Nugget and ‘Symphony’ (Gent et al., 2018) as controls and disease was rated as presence or absence.

A subset of lines presumed to be resistant to both VB, V3, V5 (isolate MN-001) and VB, V3, V4, V5, V6 (isolate HPM-666), were then inoculated with isolate HPM-204 possessing virulence on hop plants possessing R1 and R2 (VB, V1, V2, V3, V5) (Gent et al., 2018). Following the methods outlined in Gent et al. (2018), inoculations with this isolate were conducted in a class II biocontainment hood using three sets of single-node cuttings and a pair of leaves in a double Petri dish system. Inoculum was applied at 2.0×10^4 conidia mL⁻¹ using an airbrush sprayer. The number of colonies were enumerated macroscopically at 21 DPI (Wolfenbarger, Massie, et al., 2016). Each experiment was repeated at least once using a separate set of single-node cuttings.

A fourth pathogen isolate, HPM-202 (VB, V3, V4, V6, VWH18) (Gent et al., 2018) was used on a set of germplasm exhibiting no disease development following inoculation with HPM-204. This pathogen isolate is presumed to overcome the uncharacterized powdery mildew resistance found in WH18, a wild hop collected from Thuringia, Germany. Inoculations with this isolate were carried out in the same manner as isolate

HPM-204. Each experiment was repeated at least once using a separate set of single-node cuttings.

2.3 | Population assignment

As part of a larger experiment not described here, we genotyped a set of 2,595 wild and cultivated *Humulus* spp. genotypes using a genotyping-by-sequencing approach (Elshire et al. 2011). Samples were multiplexed at 96 samples per lane and sequenced across 28 lanes; additional samples were included for unrelated projects to maintain similar read depth across all samples. Raw FASTQ files were demultiplexed, trimmed, and aligned to the ‘Cascade’ reference genome (Padgitt-Cobb et al., 2022) prior to calling single nucleotide polymorphisms (SNPs). We filtered SNPs based upon read depth, missingness per sample, call rate, mapping quality, linkage disequilibrium, and Hardy-Weinberg equilibrium. We used a combination of principal coordinates analysis and K-means clustering to assign individuals to populations using a random subset of 300 individuals plus resistant genotypes.

2.4 | Sex determination and flowering time

In 2021, two replicates of each germplasm, as part of a larger experiment not described here, were established and grown under a 2.5-m trellis in a 0.4-ha hopyard in St. Paul, MN, in a Waukegan silt loam soil (Fine-silty over sandy or sandy-skeletal, mixed, superactive, mesic Typic Hapludolls). Plants were arranged in a randomized complete block design. Each plant was trained during the first week of June using a V-trellis type system with two strings per plant. Nitrogen was applied at a rate of 103 kg ha⁻¹ as urea (46–0–0 N–P–K) and broadcast across the entire hopyard. Irrigation was applied following application. Biological sex was determined following onset of flowering. Flowering time was also rated at 7-d intervals and was determined by the date of first observed inflorescence.

2.5 | Virus and viroid testing

Clonal material exhibiting resistance to HPM-202 was submitted for diagnostic testing at the Clean Plant Center Northwest (CPCNW) located at Washington State University—Irrigated Agriculture Research and Education Center in Prosser, WA. Young leaf tissue was used for total RNA extractions and subsequently subjected to reverse-transcriptase quantitative PCR (RT-qPCR) using viroid and viral specific primers for pathogen detection with positive, negative (no template) controls, and an internal *NAD5* control (Beaver-Kanuya et al., 2021; Khuu, 2021). Plants were examined for the primary viruses and viroids known to affect hop in

the United States: *American hop latent virus* (AHLV), *apple mosaic virus* (ApMV), *hop latent virus* (HLV), *hop mosaic virus* (HMV), *hop latent viroid* (HLVd), and *hop stunt viroid* (HSVd).

3 | CHARACTERISTICS

3.1 | Isolate characterization and resistance screening

The isolate selected for initial screening was subsequently referred to as MN-001 and resulted in disease on hop cultivars Yeoman, Challenger, and Early Choice, indicating the isolate possessed virulence on plants containing *RB*, *R3*, and *R5*.

We screened 6,732 seedlings and clones from 244 populations with a common pathogen race (*VB*, *V3*, *V5*) from which we identified a subset of 161 individuals (2.39%) representing 46 populations that exhibited complete resistance. These individuals were screened with an additional pathogen race (*VB*, *V3*, *V4*, *V5*, *V6*), which identified 22 individuals (13.66%) from four populations possessing resistance to two of the primary pathogenic races of *P. macularis* known in North America.

Additionally, we screened these 22 lines with a pathogen race (*VB*, *V1*, *V2*, *V3*, *V5*) not known to occur in North America by inoculation with isolate HPM-204 and identified a subset of 15 individuals (68.18%) from four populations that exhibited no signs of disease. These were further challenged with isolate HPM-202, which possesses race *VB*, *V3*, *V4*, *V6*, *VWH18*, not known to occur outside continental Europe, and identified a subset of 10 individuals (66.67%) from two populations that developed no disease following inoculation. Therefore, these ten individuals represent a set of wild hop germplasm that exhibit broad range resistance to multiple pathogenic races of *P. macularis*, representing the extant virulence on plants with previously described *R* genes.

The ten individuals identified in this study result from the collection of wild hop materials in two locations: one from the Republic of Georgia representing *H. lupulus* var. *lupulus* (two individuals, UMN-PMR-002 Male and UMN-PMR-005) and one of uncertain origin representing *H. lupulus* var. *lupulus* (eight individuals, including UMN-PMR-006 Female, UMN-PMR-007 Male, UMN-PMR-008 Female, UMN-PMR-010 Male, and UMN-PMR-013 Female).

In addition, the five individuals that were susceptible to HPM-202 but resistant to HPM-204 (*VB*, *V1*, *V2*, *V3*, *V5*) include individuals collected from both of the previous populations (two individuals and one individual, respectively), one population in Albania, representing an additional population of *H. lupulus* var. *lupulus* (one individual), and one population from Canada representing *H. lupulus* var. *lupuloides* (one individual).

3.2 | Virus and viroid testing

Of the ten germplasms subjected to viral and viroid diagnostics, seven tested negative for the six pathogens examined by RT-qPCR, one tested positive for *Hop latent virus*, and two gave inconclusive results.

3.3 | Population assignment

We identified a set of 1,004 SNPs in 2,495 individuals following demultiplexing, trimming, read mapping, SNP-calling, and filtering pipeline that adequately discriminated three botanical varieties that were included for genotyping, including *H. lupulus* var. *lupuloides*, *H. lupulus* var. *lupulus*, and *H. lupulus* var. *neomexicanus*. Using a subsample of 300 genotypes we assigned these seven pathogen-indexed accessions described in Table 1 to *H. lupulus* var. *lupulus* (Figure 1).

3.4 | Sex determination and flowering time

Of the seven pathogen-indexed plants possessing resistance to powdery mildew to HPM-202, six plants flowered during the 2021 growing season. Three of the six plants were determined to be female, and three were identified as male. There were no discrepancies between either of the two clones of the same genotype when both flowered.

3.5 | Deposition and release of powdery mildew resistant wild hop

Following the identification of the seven pathogen-indexed germplasm, clonal material was deposited in the USDA-ARS National Clonal Germplasm Repository (NCGR) in Corvallis, OR. These seven germplasm represent one individual with unknown sex (UMN-PMR-005), three that were identified as female (UMN-PMR-006 Female, UMN-PMR-008 Female, and UMN-PMR-013 Female), and three that were identified as male (UMN-PMR-002 Male, UMN-PMR-007 Male, and UMN-PMR-010 Male) (Table 1). The resistance possessed by these individuals has not been shown to exist in other wild hop populations (Seigner et al., 2006). However, recent screening conducted in unreleased male breeding lines with pathogen isolates carrying the same virulence patterns identified a set of powdery mildew-resistant genotypes (Gent et al., 2018). It remains to be determined whether this resistance is allelic or unique to those identified in this study.

4 | DISCUSSION

Previous research on screening for powdery mildew resistance in hop germplasm has emphasized the evaluation of

existing male breeding lines and female cultivars (Gent et al., 2018; Henning et al., 2017). Furthermore, limited studies have investigated the use of wild hop germplasm for the identification of powdery mildew resistance (Seigner et al., 2006). Similar to these previous studies, we identified a limited proportion of hop plants possessing resistance to one or more of the pathogen races tested and a larger proportion of them of primarily Eurasian origin (Seigner et al., 2006; Gent et al., 2018). Previous reports using wild hop germplasm used a similar sampling structure (half-sibling or bulked seed) when testing for powdery mildew resistance, and similar to their results, ours suggest the possibility of multiple different *R* genes, different combinations of *R* genes, or novel *R* genes present in these materials, though inheritance and segregation studies are warranted to fully decipher the genetic basis for this trait. Separate from previous results, we identified populations from the Republic of Georgia, which may possess unique resistance from that previously identified by Seigner et al. (2006).

Pedigrees of previously reported breeding lines are also suggestive of the putative origin of different powdery mildew resistance genes being derived from either North American or Eurasian origin (Darby, 2007; Gent et al., 2018). Specifically, there are an abundance of male breeding lines putatively possessing *R1/R2*-based resistance and containing native North American ancestry (predominantly *H. lupulus* var. *neomexicanus*), whereas those evaluated as possessing resistance to all of the pathogen isolates tested are mostly of European ancestry (Gent et al., 2018). These results are consistent with our own, where *H. lupulus* var. *lupulus* populations appear to be reservoirs of powdery mildew resistance. Of additional interest is the lack of these resistant male breeding lines in the pedigrees of currently grown commercial cultivars, which may be an indication of their performance as breeding parents or emphasis on a specific set of traits not contained in these materials (Gent et al., 2018).

While the exact nature of resistance to this devastating disease remains unknown, this subset of germplasm represents a novel resource for resistance in hop to powdery mildew. Furthermore, the pyramiding of this resistance with current extant sources could help to mitigate continued losses incurred by hop growers. Future research will be required to determine if these genotypes carry one or more genes for resistance and whether they are allelic to extant sources of resistance.

5 | CONCLUSION

The identification and release of seven new hop germplasms, maintained as virus and viroid-tested rootstock, containing resistance to the predominant pathogen races of *P. macularis*

TABLE 1 Phenotypic characteristics including powdery mildew response, biological sex, flowering time, and virus/viroid test status of seven wild hop (*Humulus lupulus* L.) accessions deposited in the USDA-ARS National Clonal Germplasm Repository

Genotype	Germplasm names	Population	Sex	Flowering traits ^a		Podosphaera macularis isolate ^b					Virus/viroid diagnostic screening ^c				
				time (day of yr ± SD)		MN-001 (VB,3,5)	HPM-666 (VB,3,4,5,6)	HPM-204 (VB,1,2,3,5)	HPM-202 (VB,3,4,6,WH18)	AHLV	ApMV	HLVd	HLV	HMV	HSVd
PI 699936	UMN-PMR-002 Male	<i>H. lupulus</i> var. <i>lupulus</i>	M	232 ± 0		-	-	-	+/-	-	-	-	-	-	-
PI 699937	UMN-PMR-005	<i>H. lupulus</i> var. <i>lupulus</i>	ND	ND		-	-	-	-	-	-	-	-	-	-
PI 699938	UMN-PMR-006 Female	<i>H. lupulus</i> var. <i>lupulus</i>	F	232 ± 0		-	-	-	-	-	-	-	-	-	-
PI 699939	UMN-PMR-007 Male	<i>H. lupulus</i> var. <i>lupulus</i>	M	228.5 ± 4.95		-	-	-	-	-	-	-	-	-	-
PI 699940	UMN-PMR-008 Female	<i>H. lupulus</i> var. <i>lupulus</i>	F	246 ± 0		-	-	-	-	-	-	-	-	-	-
PI 699941	UMN-PMR-010 Male	<i>H. lupulus</i> var. <i>lupulus</i>	M	246 ± 0		-	-	-	-	-	-	-	-	-	-
PI 699942	UMN-PMR-013 Female	<i>H. lupulus</i> var. <i>lupulus</i>	F	235.5 ± 4.95		-	-	-	-	-	-	-	-	-	-
'Brewer's Gold'	USDA No. 19001		F	197 ± 0	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
'Chinook'	USDA No. 21226		F	179.5 ± 14.85	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
'Comet'	USDA No. 62013		F	179.5 ± 14.85	+/-		ND	ND	ND	ND	ND	ND	ND	ND	ND
'Lubelska'	USDA No. 21113		F	221.5 ± 4.95	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND
'Ultra'	USDA No. 21484		F	172.5 ± 4.95	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND

Note. AHLV, *American hop latent virus*; ApMV, *apple mosaic virus*; F, female; M, male; HLV, *hop latent virus*; HLVd, *hop latent viroid*; HMV, *hop mosaic virus*; HSVd, *hop stunt viroid*; ND, not determined. Biological sex and flowering time were assessed under field conditions in 2021 in St. Paul, MN. Five hop cultivars that were included in field comparisons are presented along with the seven wild hop accessions.

^aPhenotypic expression of biological sex.

^bPhenotypic response following inoculation with *Podosphaera macularis* isolates with varying pathogenic races. - indicates an incompatible (resistant) response; + indicates a compatible (susceptible) response; +/- indicates the presence of sparse colonies.

^cPathogen status determined by reverse transcriptase quantitative polymerase chain reaction. - indicates a negative test; + indicates a positive test; +/- indicates an inconclusive result.

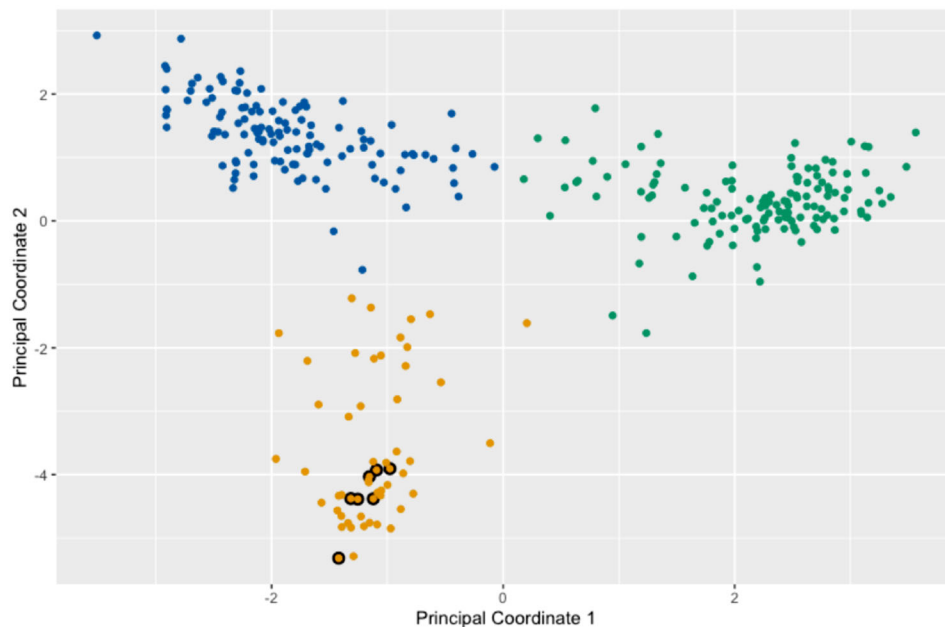


FIGURE 1 Principal coordinate analysis with populations determined using K-means clustering with $K = 3$ populations. blue, *Humulus lupulus* var. *lupuloides*; green, *Humulus lupulus* var. *neomexicanus*; orange, *Humulus lupulus* var. *lupulus*; black outlined circles, UMN-PMR-002 Male, UMN-PMR-005, UMN-PMR-006 Female, UMN-PMR-007 Male, UMN-PMR-008 Female, UMN-PMR-010 Male, and UMN-PMR-013 Female

occurring in the Pacific Northwest and elsewhere provide a valuable resource for the hop breeding community. Six of the seven germplasms had their biological sex determined, allowing for breeders to determine the direction of the cross required to introgress these resistance alleles into their elite breeding germplasm. This material is released to the public in order to address the growing need to identify and expand upon sources of powdery mildew resistance to combat this devastating disease.

6 | AVAILABILITY

Clonal material has been deposited into the National Plant Germplasm System at the USDA-ARS National Clonal Repository in Corvallis, OR. This material is available immediately upon publication for research purposes, including the development and commercialization of new hop cultivars. It is requested that appropriate recognition be given if this germplasm contributes to the development of a new breeding line or cultivar. Requests should be made to Dr. Lauri Reinhold, Curator, USDA-ARS NCGR, Corvallis, OR 97331.

AUTHOR CONTRIBUTIONS

JSH, DHG, and GJM conceived the experiments; JSH collected and gathered germplasm used for experiments; JSH, MSW, and DHG carried out the investigations; JSH, JAH, and GJM secured funding for the project; JSH conducted the formal analysis and drafted the initial manuscript; JAH, DHG

and GJM provided supervision; JSH and GJM were responsible for project administration; all authors reviewed and edited the manuscript; all authors approved the final manuscript. Joshua S. Havill: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Visualization; Writing – original draft; Writing – review & editing. Michele S. Wiseman: Investigation; Writing – review & editing. John A. Henning: Funding acquisition; Resources; Supervision; Writing – review & editing. David H. Gent: Conceptualization; Investigation; Methodology; Supervision; Writing – review & editing. Gary J. Muehlbauer: Conceptualization; Funding acquisition; Project administration; Resources; Supervision; Writing – review & editing.

ACKNOWLEDGMENTS

The authors express their gratitude to Dr. Peter Darby for providing technical advice with approaches to their experiments. The authors are grateful to Adam Schrankler and Casey McNichols for their assistance in carrying out the experiments and lastly, Yuka Kutsumi and Maxim Mallaliev for providing additional germplasm samples acquired under USDA Animal and Plant Health Inspection Service Permit to Import Plants and Plant Products (Permit # P37-19-02139). The authors acknowledge the Minnesota Supercomputing Institute (MSI) at the University of Minnesota for providing resources that contributed to the research results reported within this paper. This work was supported by funding from the Minnesota Department of Agriculture (Award No. CON000000071123)

and Hopsteiner, S. S. Steiner, Inc. JSH was also supported by fellowships from the Plant and Microbial Biology graduate program at the University of Minnesota.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORCID

Joshua S. Havill  <https://orcid.org/0000-0002-7939-9047>

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How to cite this article: Havill, J. S., Wiseman, M. S., Henning, J. A., Gent, D. H., & Muehlbauer, G. J. (2023). Registration of seven powdery mildew-resistant wild hop germplasm lines. *Journal of Plant Registrations*, 17, 171–179. <https://doi.org/10.1002/plr2.20255>