Management Strategies for Hop Downy Mildew Utilizing Fungicides and Host Resistance

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Dedication

This thesis is dedicated to my wife and daughter, who have provided smiles and kisses when needed the most. It is their unwavering love, commitment, and support that I will always carry with me.

Abstract

Hop (*Humulus lupulus* L.) is an herbaceous perennial plant species that is native to the Northern hemisphere. The hop inflorescences are utilized during the production of beer. Commercial production of hops requires the use of fungicides and host resistance to retain high overall yields and quality characteristics that brewers are satisfied with. The primary objectives of this research were to determine effective fungicidal compounds that can be used to control hop downy mildew (caused by *Pseudoperonospora humuli* (Miy. et Takah.) Wils.) and to collect and characterize a panel of 112 diverse *H. lupulus* accessions for resistance to *P. humuli*. Results from field fungicide trials indicate that significant interactions exist between cultivars, environments, and fungicidal compounds with regards to disease severity. With regards to host resistance, *H. lupulus* var. *lupuloides* E. Small accessions originating from the United States were highly-resistant to *P. humuli* compared to their North American counterparts *H. lupulus* var. *neomexicanus* Nelson & Cockerell or *H. lupulus* var. *pubescens* E. Small. Comparisons of *H. lupulus* var. *lupuloides* from Canada indicated that accessions from the United States were significantly more resistant to *P. humuli*, but not significantly different from *H. lupulus* var. *lupulus* accessions originating from Kazakhstan. These results indicate that control of hop downy mildew can be accomplished through use of resistant cultivars, fungicidal compounds, and selection of proper environments for cultivation. Additionally, utilization of *H. lupulus* var. *lupuloides* will increase the diversity of resistant sources to hop downy mildew in the development of new cultivars.

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Chapter 1

Taxonomy, Breeding, and Pest Management in Hop (*Humulus lupulus* L.)

The Genus and species

The plant family Cannabaceae contains two genera: *Cannabis* and *Humulus*. The genus *Humulus* encompasses herbaceous plants including species classified as common hop. *Humulus* is native to the Northern Hemisphere, with regions in western China reported as the center of origin (Neve, 1991). Hop species are found naturally between latitudes of 35 - 70°, in riparian environments or where significant water accumulation may occur. Three distinct taxonomic species comprise hop: *H. japonicus* Siebold & Zucc. (syn. *H. scandens* (Lour.) Merr), *H. lupulus* L., and *H. yunnanensis* Hu. Only *H. lupulus* is economically important, being grown worldwide as the primary bittering and flavoring component of beer (Neve, 1991). All three *Humulus* species occur naturally in China and Neve (1991) proposed China as the center of origin. Based on molecular evidence Murakami *et al*. (2006a; 2006b) suggested that species divergence among North American and Asian wild hops may have occurred more recently (0.46 ± 0.17 to 0.69 ± 0.21 million years ago) compared to European varieties (estimated approximately 1.05 ± 0.28 to 1.27 ± 0.30 million years ago). These findings by Murakami *et al.* (2006a; 2006b) are supported by investigations from Boutain (2014) with regards to multi-locus sequence analysis of hop specimens originating from China.

Botanical characteristics and species distributions

All *Humulus* spp. are dioecious and photoperiodic, flowering under short-day (long-night) conditions. Flowers are borne on lateral branches and are initiated during the weeks following the solstice, triggered by shortening daylengths and temperature relationships. It has been noted that flowering is greatly reduced in areas receiving less

than 15 hours of light after floral initiation, which can be circumvented in growing regions below 35° latitude with supplmental lighting (Thomas and Schwabe, 1969; Thomas and Schwabe, 1970).

The leaves are decussate and simple with serrate to doubly-serrate margins, have acuminate leaf apices and a cordate base. Leaves are generally palmate and often have numerous lobes, the features of which are also used for species and botanical variety identification (Small, 1978; Small, 1981). The male inflorescence is prolifically branched and arranged in a panicle. The flowers have five petals, in which the stamens and anthers are attached tightly. Female inflorescences (commonly referred to as cones) contain a central axis or rachis with several nodes, at each node extends a pair of bracts which loosely envelopes a pair of bracteoles. Within each bracteole, a flower forms which contains an ovary with an ovule bearing two papillated stigmata. If fertilized, the ovary will develop into an achene, the rachis increases in size and seeds develop within the bracteoles (Neve, 1991; Britton and Brown, 1910). Seed maturation occurs at the end of the season and seeds are readily dispersed via wind and rain when dislodged from the rachis.

Spring regrowth from perennial plants is dependent on the age and maturity of the plant. As the plant matures, the crown can form a few to many buds that will overwinter and initiate growth in the spring. Bines curl in a clockwise fashion around a support. *H. lupulus* and *H. yunnanensis* are perennial, forming rhizomes in the top 15 centimeters of soil. Plants also form taproots up to 3 meters in length with lateral roots that extend several meters from the stem into the top 20 – 30 centimeters of soil, though the extent of root growth observed is dependent on soil-type (Beard, 1943).

Humulus lupulus

H. lupulus (Figure 1.1, 1.2, 1.3) has a widespread distribution within North America, Europe, and Asia. Small (1978) reclassified *H. lupulus* species into five distinct botanical varieties based on variation in morphological characters, as well as distribution. Studies of *H. lupulus* indicate that 10 pairs of chromosomes are present in cells of both male and female, with a distinct heteromorphic pair of sex chromosomes (Grabowksa-Joachimiak *et al*., 2006).

H. lupulus var. *cordifolius* (Miquel) Maximowicz is native to Asia and can be recognized by morphological differences which are commonly based on the density of trichomes and climbing hairs found on the stem and leaf surfaces. *H. lupulus* var. *lupulus* has been denoted as the most widely distributed botanical variety, occurring both naturally and as an introduced plant on six of seven continents. Most cultivars in production today are also derived from hybridization with this variety due to the favorable characteristics such as agronomic traits, disease resistance, and brewing properties (Small, 1981).

Other *H. lupulus* botanical varieties are considered indigenous to North America and include *H. lupulus* var. *lupuloides* E. Small, *H. lupulus* var. *pubescens* E. Small, and *H. lupulus* var. *neomexicanus* A. Nelson & Cockerell. *H. lupulus* var. *lupuloides* (Figure 2) is found growing in north-central and eastern regions of North America and can be identified by the general lack of trichomes (<100/cm), high density of lupulin glands (>25/cm^2), and possession of generally fewer than five lobes on floral leaves. *H. lupulus* var. *pubescens* (Figure 3) is found growing in south-central regions of the United States,

and has large densities of trichomes (>100/cm) on all surfaces of the plant, high lupulin gland density (>25/cm^2), and fewer than five lobes on floral leaves. *H. lupulus* var. *neomexicanus* (Figure 3) is found growing in the Pacific Cordilleran regions. General morphological features include pronounced leaf margins, increased glandular deposition, and increased lobe number on floral leaves (Small, 1978; Small, 1981).

Molecular evidence differentiates North American hops from Eurasian hops, but fewer studies have focused on delineating the relationships amongst North American wild hops (Murakami *et al*., 2006). Smith *et al*. (2006) suggest that greater genetic and phenotypic diversity is present in *H. l.* var. *neomexicanus* as a result of recent and repeated glaciation. Tembrock *et al.* (2016) provide evidence that supports the elevation of these three botanical varieties to species based on defined morphological characters and recent studies determined a lack of evidence for gene flow between sympatric populations (Richards and Reeves, 2011). Boutain (2014) differentiates *H. lupulus* into two primary groups, those from Eurasia and those from North America, each with sub-groups which correspond readily with geographic origins. Interestingly, whether taxonomic relationships can be delineated within *H. lupulus* or not, all five botanical varieties can be inter-hybridized, although no commercial cultivars contain *H. l.* var. *pubescens* within their pedigree. This may be due to poor availability of specimens present in current germplasm collections.

Humulus japonicus

H. japonicus is an annual plant. It is easily distinguished from other species by the high number of leaf lobes, generally five to nine, as well as the stout, hooked hairs

present on the stems and leaves. Another major distinction is the reduction or lack of lupulin glands in *H. japonicus*. Cytological investigations have shown that female and male are distinguishable by the number of chromosomes, having 16 or 17, respectively (Grabowska-Joachimiak *et al*., 2006). *H. japonicus* is native to areas in China and Japan, and is considered invasive in regions such as North America where it has been introduced as an ornamental species.

Humulus yunnanensis

H. yunnanensis is distributed throughout southern China in the Yunnan Province. It is a perennial plant with palmate leaves generally being three to five lobed. Floral characteristics are similar though distinct from *H. lupulus*, having only one pair of flowers instead of two at each node of the inflorescence. The adaxial and abaxial leaf surfaces are more densely pubescent compared to either of the other two species (Small, 1978; Wu *et al*., 1994). Boutain (2014) delineates genetic relationships of *H. yunnanensis* to other *Humulus* spp. and provides additional evidence to support claims made by Small (1978) that *H. yunnanensis* is a distinct species. To date there is still an appreciable lack of evidence concerning this species and its biology.

History and uses of hops

Originally, beer was flavored with herbs and spices like bog myrtle, wild rosemary, ginger, sage, and mint. The value of hops lies primarily in the production and extraction of bitter resins and oils that contribute characteristic bitterness, flavor, and aroma to beers. Initial hop usage for beer making was due to the anti-microbial

properties it contributed to beer. Hops decreased the incidence of spoilage and increased the length of time beer could be stored compared to beers created with other plant materials (Moir, 2000). Hops have also been utilized for their medicinal properties and extracts have been recognized as having anti-proliferative effects on cancerous or tumorigenic growths (Delmulle *et al*., 2006; Ho *et al*., 2008).

The first recorded cultivation of *Humulus* spp. occurred in Bavaria in the eighth to ninth centuries (Linke and Rebl, 1950; Neve, 1991). It wasn't until the twelfth and thirteenth centuries that large quantities were noticed within fossil records of continental Europe (Wilson, 1975). As hop cultivation and use in brewing became more popular, the Bavarian Purity Law was enacted in 1516. This law enforced the use of only hops, malted grain and water in the production of beer. In 1524, the Dutch were recruited to aid in the establishment of hopyards in England, which had been importing hops for brewing. Material recovered from sailing vessels established the presence of hop in southeastern England during the tenth century (Wilson, 1975).

In 1629, the Massachusetts Company introduced the hop plant into settled areas along the Eastern coast of North America for the domestic production of beer. The first commercial North American hopyard was established in 1808 in New York, with production growing for decades and then collapsing due to a number of factors, including plant disease pressures, prohibition, and environmental conditions (Burgess, 1964). Furthermore, these factors helped push hop cultivation further west towards areas in the Midwest such as Minnesota and Wisconsin, as well as the Western states consisting of California, Idaho, Oregon, Washington (Burgess, 1964; Hibbard, 1904; Neve; 1991; Schwartz, 1973). Subsequent deterioration of commercial hop-production in the Midwest

during the late 19th and early 20th century was reported to be influenced by a number of factors, especially insects and disease (Dodge, 1882).

Expansion of hop cultivation in other regions of the world including Australia, New Zealand, South Africa, Japan, and India continued for the next several decades, primarily due to the increasing demand for beer (Neve, 1991; Simmonds, 1877). Due to the long photoperiod required for optimal yields, some regions have adapted cultural practices to provide supplemental lighting for successful cultivation (De Lange *et al.*, 2015). Areas where such issues arise also generally cannot induce plant dormancy, as would be provided in more temperate regions that have a cold season and are capable of meeting the vernalization requirements of the plants.

During the past decade, hop cultivation has been re-introduced to the Midwest. An increasing number of breweries and market trends favoring locally-produced ingredients have aided in the establishment of organizations such as the Minnesota Hop Growers Association, the Wisconsin Hop Exchange, the Michigan Hop Alliance, and the Nebraska Hop Growers Association (Moskowitz-Grumdahl, 2014). Currently cultivar trials and production methods are being researched by several institutions to determine the viability of a hop-growing industry across different regions (Turner *et al.*, 2011; Pearson *et al*., 2016).

Production, cultural, and pest management strategies

Hops are grown commercially on numerous soil types, in vastly different environments worldwide. Both climatic factors and soil types impact the overall growth and development of the plant. Mahaffee *et al*. (2009) describe soil types in cultivated

production as including ‘deep alluvial loams, slight to moderately calcareous eolian silts, and clay-loam soils derived from lacustrine deposits’. In general, commercial hops production requires sufficient soil moisture, either through rainfall or irrigation as well as adequate drainage. Soil pH is also important, as this impacts the availability of many important nutrients in the soil. Ranges from pH 5.8 – 7.5 are capable of providing most nutrients in sufficient concentrations, though a pH of 6.5 is optimal (Mahaffee *et al*., 2009).

Nutrient management is an important aspect of plant health, and while recommendations are generally made based on existing soil information, one must take into account several aspects including soil types and soil pH, the need for irrigation, and potential leaching capacity of the fertilizers being used. Nitrogen can be managed through both fertilizer addition as well as subsequent return of crop debris to the soil after harvest. Potassium and phosphorus needs can be managed with proper applications of fertilizers based on preliminary soil analyses to allow for ideal application rates. Proper and uniform composting of plant debris after harvest, before returning to the field from which it was taken can also reduce the incidence or build-up of pathogens over time (Bailey and Lazarovits, 2003) while also improving soil structure and health and maintaining yields (Liu *et al*., 2013).

Irrigation of plants is necessary to achieve optimal yields and consistent quality, especially in areas with particularly arid climes. Yield is impacted by water deficiencies manifesting as a loss of overall vegetative growth, resulting in decreased lateral growth and subsequent flowering (Nelson *et al*., 1966). Water deficiencies have no discernable effects, however, on overall brewing quality of the cones (Nakawuka, 2013).

Hops require significant physical infrastructure for proper management. Standard practice includes trellising on a permanent structure made of vertical poles with horizontal cables stretched across the top of adjacent poles. Coir twine is attached from the ground to the cables to provide a rough surface for the hop bines to readily climb. The height of the trellis is generally dependent upon the method of cultivation and the grower's capabilities. Trellising generally ranges from 4 – 8 m in height, though in the United States most trellising is between 5 – 6 m. Often, in the United States, stringing is performed from a raised platform as workers pass beneath the top wire and tie or hook the premeasured twine. Later on, the twine is secured in the ground with a spike (Neve, 1991).

Plant spacing has varied depending on cultivation methods and equipment size. Cultivation of hops can include close plantings with 1.6 m between rows and 1.4 m between plants within a row and one twine per plant. The increased use of mechanical implements for large acreages has required wider spacing, typically either 3 m between rows and 1.5 m between plants within a row or 2.3 m by 2.3 m. Regardless of spacing methods, growers typically provide two to four strings per plant. Several studies have concluded that higher planting densities produce higher yield potential per acre, while lower plant densities result in larger per plant yield potentials (Stranc *et al*., 1979). Additional factors such as trellis height, number of bines trained to grow on a string, irrigation, and nutrient applications can significant impact yield potential (Keller and Li, 1951; Keller and Magee, 1952; Koren, 2007; Nakawuka, 2013; Wample and Farrar, 1983).

Although the cable and coir system described above is popular with commercial hops producers, other cultivation methods exist. Recent work aimed to understand the implications of "low-trellis" production on the yield and health of hops. A low-trellis system entails use of a permanent mesh installed between the poles allowing, the spring regrowth to train itself upon the mesh. In this system, the hops plants remain permanently attached to the mesh, and inflorescences are harvested, in the field, without cutting the plant. This practice is more common in England due to the availability of specially constructed harvesting machinery adapted to low-trellis production. Four major issues arise from this production method, including labor intensive nature of harvesting, difficulty in perennial weed management, stability of the structure over time, and potentially greater disease severity with residual plant debris. These latter two issues could result in crop loss if the mesh is not replaced in a timely manner, as the resulting weight of the hops plants over consecutive years could displace the entire structure or residual crop debris incites disease epidemics.

At the end of the growing season, plants are cut several feet above the ground, coir and bines are detached from the top wire, and the severed vines are run through a mechanical harvester that strips and sorts the inflorescence from bine, leaf, and stem material. The inflorescences are placed into an oast, a drying unit in which successive layers of hops, of varying moisture content, can be placed at different levels and are continually monitored and rotated through until the optimal drying is achieved (Neve, 1991).

Two main groups of soft resins occur together within the growing and mature inflorescence and are referred to as α- and β-acids that together impart the antimicrobial

and bitter properties to beer. Humulone, cohumulone, and adhumulone make up the α-acid fractions; each is distinguished by their alkyl side-chains which contain isobutyl, isopropyl, or sec-butyl side-chains, respectively. Lupulone, colupulone, and adlupulone are the constituents of the β-acid fractions (Nickerson *et al*., 1986). Each variety has a generally accepted range of values for these characters though consistency is not always achieved due to environmental conditions or regional differences (Mozny *et al*., 2009). Additionally, hop varieties are generally categorized by their brewing characteristics, usually into one of two groups: aroma or bittering hops. Aroma hops are generally regarded as having low bittering potential, usually < 6% α-acids in w/v, whereas bittering varieties are associated with higher amounts of α-acids but may not have the desirable oil profile.

Commercial hop production generally includes the production of unfertilized flowers as resources within the plant are diverted from the production of desirable chemical compounds (i.e. essential oils and soft resins) after fertilization. However, harvesting unfertilized cones leads to a decrease in yield, on a per weight basis (Hartley, 1965; Thomas and Neve, 1976). Previous work has shown that the distribution of α-acids, a group of soft resins containing bitter compounds, within the growing inflorescence is also dependent upon fertilization. Specifically, seeded cones accumulated larger amounts of α-acids on the seed coat compared to unfertilized flowers, which produced these fractions primarily within the bracts and bracteoles (Neve, 1968). Initially, brewers were concerned about the contribution of fatty acids and other lipid compounds to beer quality and stability but subsequent determination of the effect of

seeded hops in beer indicated that there was little to no effect on beer quality (Harrison, 1971).

Numerous pest management tactics have been employed to reduce disease severity in commercial production. These primarily include several applications of pesticides throughout the growing season, followed by additional release of bio-control organisms and use of cultural practices that reduce transmittance of pathogens within and between hop-yards. Of particular importance is the breeding of disease resistance or tolerance, and there are numerous accounts of successful deployment of improved cultivars with increased resistance. Brewer preference is an important factor to consider in breeding as recent emphasis on commercializing resistant cultivars has resulted in brewers opting instead for disease susceptible varieties with better brewing attributes. This is especially true for craft brewers, who often have an interest in less-widely grown (and often more susceptible) cultivars or landraces (Salmon, 1930; Woods and Gent, 2016).

Hop downy mildew

Pseudoperonospora humuli, the causal organism of hop downy mildew, is a homothallic oomycete pathogen and obligate biotroph. First described from diseased hop tissue in Japan by Miyabe and Takahashi (1905) as *Peronoplasmopara humuli* n. sp. and later revised by Wilson (1914), *P. humuli* was first identified in North America in Wisconsin on wild hops in 1909 (Mitchell, 2010; Miyabe and Takahashi, 1906; Skotland and Romanko, 1964). *Pseudoperonospora humuli* primarily reproduces asexually through sporangia in a polycyclic manner throughout the growing season. These

sporangia are disseminated via air and water. Additionally, sporangia are stimulated to release zoospores following a sufficient length of exposure to water. Zoospores encyst singly over open stomata forming a germ tube and subsequent sub-stomatal hyphae during the early phases of infection (Royle and Thomas, 1971a; 1971b; 1973; Pares and Greenwood, 1977; 1981). Research performed by Ware (1926, 1929), Coley-Smith (1962b, 1964), and Skotland (1961) provided definitive evidence of the contribution of mycelia to the disease cycle. Their results indicate that primary inoculum of *P. humuli* arises from systemically infected shoots which form as dormant buds in the preceding season.

While oospores can be found in necrotic tissues, their overall importance in the epidemiology and life cycle of *P. humuli* has yet to be determined. Disputes over the ability of the oospore to germinate *in vitro* have limited progress in determining its contributions to the disease cycle (Arens, 1929; Bressman and Nichols, 1933; Gent *et al*., 2017; Mitchell, 2010; Parker, 2007). Skotland and Johnson (1983) suggested environmental and host-specific cues may significantly influence the outcome of oosporic inoculum, though several thorough bioassays performed by Gent *et al*. (2017) were unable to stimulate oospore germination.

By the late 1920's, additional hop growing regions including British Columbia, England, Germany, and the Pacific Northwest reported downy mildew. One explanation for the rapid expansion of the distribution of the disease may be the movement of plant materials across international borders during establishment of regional hop breeding programs. Whether or not *P. humuli* is a native or introduced pathogen, wild hops may

potentially serve as an inoculum source, allowing new pathogen strains to arise independent of commercial production (Claassen *et al.*, 2017).

Symptoms and epidemiology

Systemically infected shoots referred to as "basal spikes" emerge in spring following the cessation of dormancy. These infected shoots display stunted growth and symptoms of chlorosis which radiate from the base of the leaf blade outwards towards the leaf margins. Infection of the perennial hop crown can lead to unevenly distributed infections of the crown buds, with both healthy and infected shoots arising from the same plant (Coley-Smith, 1962; Ware, 1926). Sporulation occurs on the abaxial surface with sporangiophores emerging in the early morning hours under favorable environmental conditions (Royle, 1970; 1973; Royle and Kremheller, 1981; Royle and Thomas, 1973). Dispersal of sporangia through rain or wind allow for subsequent secondary infections. Leaf infections are visible as angular chlorotic lesions, bounded by leaf veins, often coalescing in highly susceptible cultivars. Secondary shoot infections may occur on main or lateral shoots producing "aerial spikes", which lead to declines in overall cone yield or quality. Following systemic infection of the plant, the crown rot phase of the disease can occur but is thought to primarily be a concern in areas not conducive to the foliar phase of the disease (Royle and Kremheller, 1981; Skotland, 1961). The foliar phase of the disease primarily functions as a mechanism of secondary spread of the pathogen between plants in a hopyard. The crown rot phase is primarily associated with production of basal spikes or crown death, which may occur in highly susceptible cultivars (Royle and Kremheller, 1981; Woods and Gent, 2016). Lastly, infection of the cone is of primary

concern, due to issues resulting from loss of yield or quality. Discoloration of the cone can result in rejection of the entire crop by a brewer (Royle and Kremheller, 1981).

Hop breeding and resistance to hop downy mildew

For centuries in continental Europe, the hop was continually selected from landraces and propagated asexually via rhizomes. There were likely hundreds of different plants, each genetically distinct from another, included in early hopyards. These early hop genotypes were selected for acceptable growth, vigor, and yield, while maintaining specific brewing characteristics and only those exhibiting the desired traits were propagated and replanted. It was commonplace for a grower to subsequently attach their name to a variety for which they were reputed. The cultivar Fuggle, selected by Richard Fuggle in 1861, is a prime example (Parker, 1934). Additional cultivars, such as 'Goldings', 'Hallertauer', 'Saazer', 'Spalter', and 'Tettnanger' were named based on the region or person with which they were associated. Many clonal selections, usually landraces, are classified as aroma hops, while many of the subsequent hybrids between American and European cultivars are bittering hops.

Intentional breeding programs were initiated in both Germany and the United States in 1894 but little progress was accomplished initially. In 1904, the Wye College in England established a hop breeding program, having accumulated a large collection of plants over the decade prior for use as founders for improvement. This germplasm was recovered from North America, England, and continental Europe. Within the Wye breeding program cultivars such as 'Wye Challenger', 'Wye Northdown', 'Wye Target', 'Wye Yeoman', and 'Wye Zenith' were released and touted as being highly resistant to

hop downy mildew, in addition to containing resistance to other pathogens (Haunold, 1981; Neve, 1991). The Wye breeding program was diverse in breeding methods and germplasm use, compared to those in Czechoslovakia, Denmark, and Germany, which primary emphasized clonal improvement and selection (Neve, 1991).

The outbreak of downy mildew in Germany in the 1920's, followed by the subsequent introduction of downy mildew into the United States crystalized the need for breeding programs to focus efforts towards breeding disease-resistant varieties (Zattler, 1928; Zattler, 1931). Similar refocus on disease resistance breeding occurred again in the 1960's when Verticillium wilt (*Verticillium albo-atrum* and *Verticillium dahliae*) was introduced into Germany. Today, hops breeding efforts in Australia, China, Germany, Japan, New Zealand, Russia, Slovenia, South Africa, and the United States emphasize breeding for both pathogen-specific disease resistance and market demands.

Several techniques useful in generating significant genetic and phenotypic variation while maintaining useful characteristics have been applied to hop breeding. Several breeding programs have utilized anti-mitotic agents, such as colchicine or oryzalin, to create polyploid breeding lines useful in generating triploid cultivars. The triploid state offers two benefits: infertility as a result of uneven meiotic division and a genetic background containing two-thirds of the requisite cultivar upon which improvement is sought. Triploidy reduces the incidence of seed set, which might be common in regions where males were either mistakenly or purposefully planted, and increases the chance of progeny containing the desired characteristics of the parent generation due to potential masking of deleterious alleles, higher gene expression, or gene dosage effects. It has also been noted that there is a high relative frequency of

mixoploid cell types that arise in polyploid individuals (Beatson *et al*., 2003). Previous research has indicated that while mixoploid tissue is useful in recovering additional polyploid plants through tissue culture methods there are no significant differences between the different grades of mixoploid tissue in their ability to regenerate polyploids (Beatson *et al*., 2003; Neve, 1991; Roy *et al*., 2001; Zattler, 1960). Some cultivars are the result of spontaneous mutations within meristematic tissues. Hop genotypes seem to differ in the frequency of spontaneous mutations and are common in genetic backgrounds including 'Golding', 'Cluster', 'Kirin II', 'Saaz', and 'Talisman'. Mutant genotypes arising from these genetic backgrounds include those that mature at different times, contain different chemical profiles than the parental variety, or specific morphological changes such as dwarf stature (Neve, 1991; Patzak, 2003). Neve (1991) also discusses the impact that clonal selection has had on the prevalence of viruses and how viruses may have contributed to phenotypic variation between related clonal populations.

A number of studies have attempted to quantify the phenotypic and molecular variation present among and between current cultivars and to assess the variation among wild accessions or determine their usefulness as breeding material (Brady *et al*., 1996; Cerenak *et al*., 2005; Henning *et al*., 2004; Jaske *et al*., 2004; Jaske *et al*., 2008; Kavalier *et al*., 2011; Murakami *et al*., 2006a; Pillay and Kenny, 1996; Seigner *et al*., 2008). Further discussions by Henning (2006; 2012) about the past, present, and future application of these technologies to hop improvement detail both the pitfalls and benefits that each has had in hop research. Marker-assisted selection in hop has been limited by gaps in understanding the genetic architecture of traits of interest. Unlike in some better studied crops, efforts to identify quantitative trait loci (QTL) that influence important

traits have yet to contribute significantly to the development of new hop cultivars (Cerenak *et al.*, 2006; Cerenak *et al.*, 2009; Bernardo, 2008).

Research Summary

Expansion of hop production into the Midwest presents need and opportunity to pursue disease management research to control downy mildew of hop. The objectives of this thesis were to examine the use of fungicides (Chapter 2) and host resistance (Chapter 3) as an effective mechanism for disease management. Specifically, this work aims to establish baseline recommendations for commercial fungicide applications in the Midwest and to identify novel genetic resistance to *P. humuli* from native and non-native populations of *Humulus* spp. useful for development of cultivars adapted to the Midwestern United States.

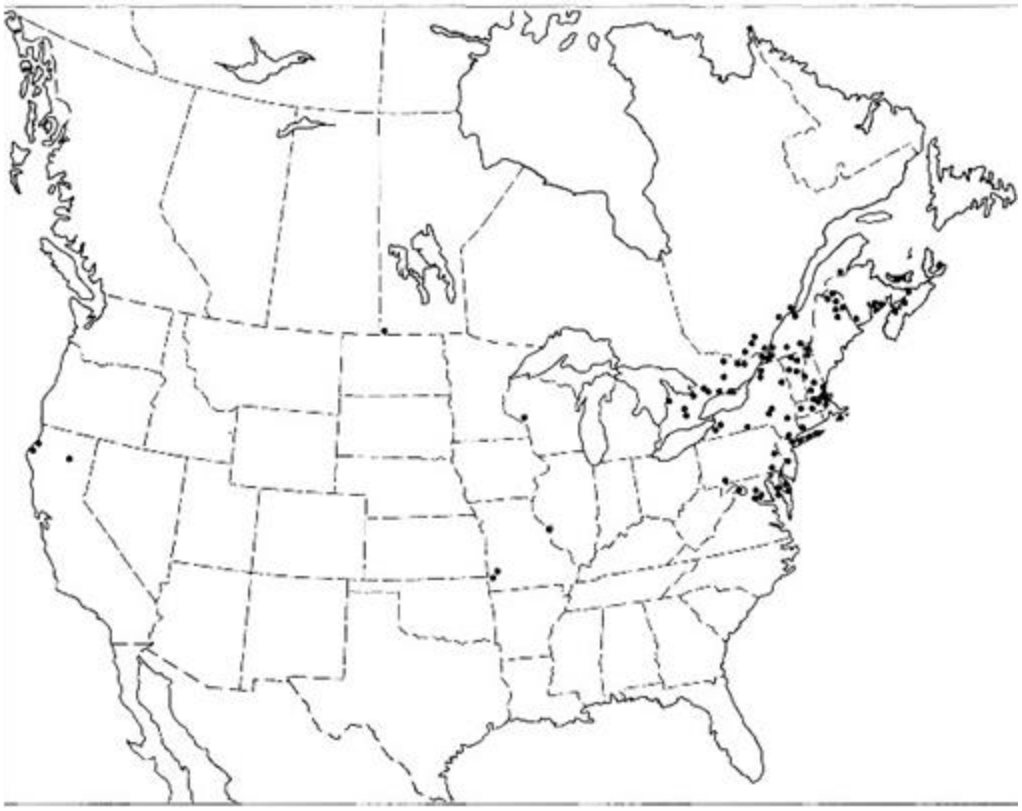

Figure 1.1. Introduced range of *H. lupulus* var. *lupulus* populations. Black dots represent herbarium records. (Small, 1978).

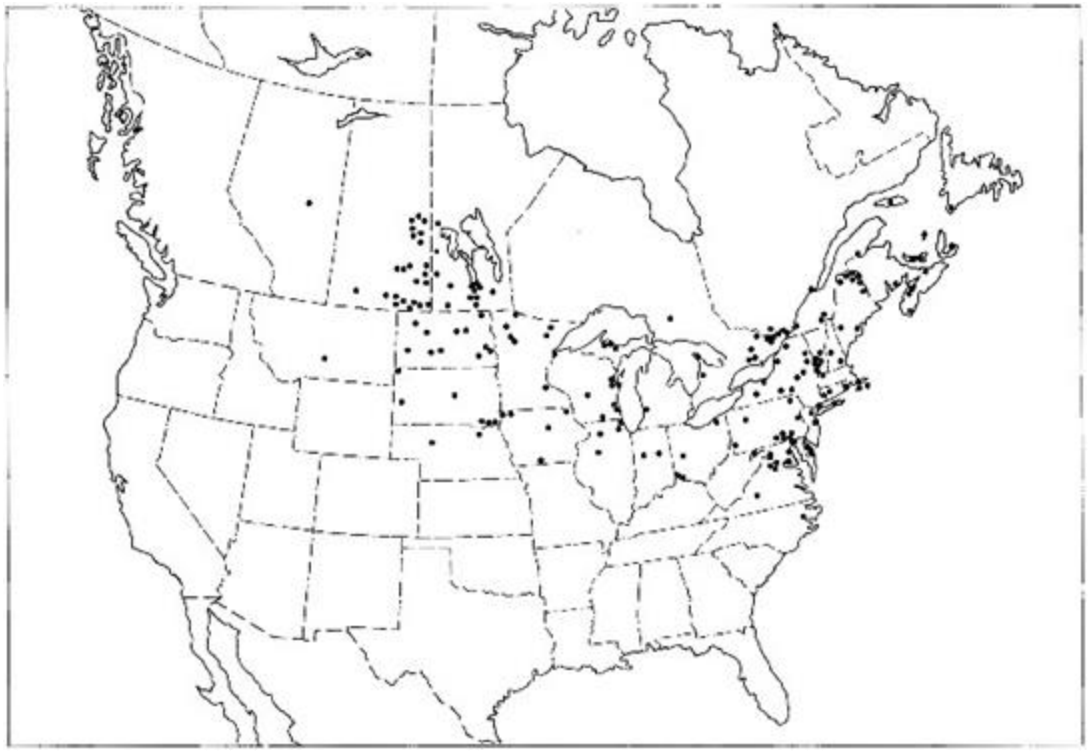

Figure 1.2. Natural range of *H lupulus* var. *lupuloides* populations. Black dots represent herbarium records. (Small, 1978).

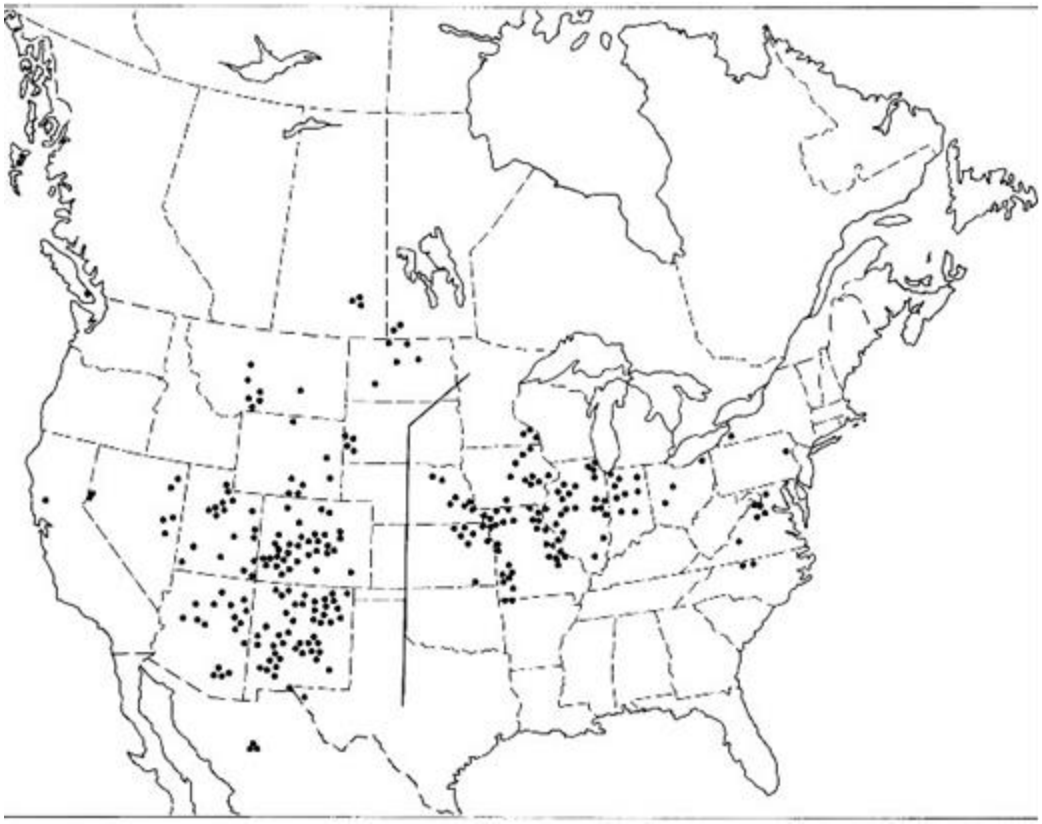

Figure 1.3. Natural range of *H. lupulus* var. *neomexicanus* (left) and *H. lupulus* var. *pubescens* (right). Black dots represent herbarium records. (Small, 1978).

Chapter 2

Fungicide Efficacy in Midwestern Hop (*Humulus lupulus* L.) Production

Summary

Pseudoperonospora humuli Miy. et. Takah., the causal organism of hop downy mildew, is a major pathogen of hop (*Humulus lupulus* L.). Commercial hop production is highly dependent upon multiple fungicide applications as the primary control method for hop downy mildew. Although tolerant varieties do exist they are not commonly planted and this further necessitates the use of fungicides in regions extremely conducive to the disease, such as the Midwestern United States. In this study, we describe research field trials conducted during 2015 and 2016 in Minnesota to evaluate the efficacy of fungicide programs on two hop cultivars. Disease severity varied significantly across locations and fungicide treatments. Only the fungicide program containing Tanos® significantly reduced disease severity in field trials during 2016. Additionally, *in vitro* examination of fungicides commonly registered for use in commercial hop production to control downy and powdery mildew (*Podosphaera macularis* Braun & Takam.) were assessed using a detached leaf assay. Fungicide treatments significantly reduced percent diseased leaf area, with fluopicolide (Presidio®) having the greatest overall percent reduction in percent diseased leaf area. Due to the short growing season in Minnesota certain cultural practices such as crowning may not be feasible, which means reliance upon fungicides should be considered as the main method for controlling incidence and severity of hop downy mildew.

Introduction

Hop downy mildew, caused by *Pseudopersonospora humuli*, is a devastating disease of hop (*Humulus lupulus*). It is ubiquitous in all regions where hop is grown, except in Australia, New Zealand, and South Africa (Neve, 1991). There are several chemical and cultural practices that are utilized to reduce the damage caused by hop downy mildew. The most commonly planted varieties in the United States are often the most susceptible to the disease and include cultivars such as Cascade, Centennial, Chinook, and Columbus (Gent *et al*., 2010; Gent *et al.* 2012b). One of the primary reasons hop cultivars with increased downy mildew tolerance are not utilized is they lack desirable agronomic or brewing characteristics (Woods and Gent, 2016).

Plants infected by *P. humuli* exhibit multiple symptoms depending on the phase of the disease. Early symptoms associated with systemic infection by *P. humuli* are chlorotic, stunted shoots which are commonly referred to as "basal spikes". These systemically infected shoots provide the primary inoculum and allow for early season spread of the disease within a hopyard. Subsequently, vigorously climbing apical shoots or lateral branches can become infected and develop into "aerial spikes", which allow for secondary spread within the dense plant canopy. The hop inflorescence, also referred to as the hop cone, can become infected which can lead to additional reductions in both cone yield and quality (Gent *et al*., 2012a; Gent *et al*.2012b; Johnson *et al*., 2009). Hop crowns can also become infected, primarily through mycelial invasion of the dormant buds that form on the crown (Coley-Smith, 1962b; 1964; Skotland, 1961). This crown rot phase reduces the availability of carbohydrates in the plant roots and rhizomes and

can lead to plant death in highly-susceptible varieties (Coley-Smith, 1964; Skotland, 1961; Williams *et al.*, 1961; Woods and Gent, 2016).

Cultural sanitation practices used in reducing hop downy mildew include removing infected growth and debris. This can be performed mechanically or chemically and is commonly used to control early season disease (Gent *et al*., 2012b; Gent *et al*., 2015, Probst *et al*., 2016). Prophylactic fungicide treatments are the most efficacious method in limiting establishment of *P. humuli* but in areas where weather factors favor disease development this practice can significantly increase production costs (Gent *et al*., 2010; Gent *et al.*, 2015). Indiscriminate use of fungicidal compounds also creates the potential for fungicide insensitivity to develop within the pathogen population. Insensitivity to fosetyl-Al and metalaxyl (syn. mefenoxam) has been identified in traditional production regions of the Pacific Northwest in the states of Idaho, Oregon, and Washington (Gent *et al*., 2008; Hellwig *et al.*, 1991; Klein, 1994; Nelson *et al*., 2004). Newer regions of production which have sourced cultivar planting materials from a diverse range of locations, including the Pacific Northwest, may have introduced these insensitive strains (Wolfenbarger *et al*., 2016; Marks and Gevens, 2016).

Establishing fungicide application recommendations for commercial hop producers that are tailored to unique regional environments is integral to the successful resurgence of hop production in new production areas, including the Midwest. Commonly, field studies combined with greenhouse or *in vitro* assays are used to determine efficacy and support recommendations. All methods of assessment are utilized to determine effective application rates and percent disease control with *P. humuli*. Our objective in this study was to determine fungicide efficacy of commonly applied

fungicides in Midwestern hop production in the field and to evaluate an expanded set of fungicidal compounds using an *in vitro* assay.

Materials and Methods

Field plot setup. In July 2014, 0.10 acre hopyards were established at three University of Minnesota Research and Outreach Centers (Grand Rapids, 47.246 °N, -93.494 °W; Rosemount, 44.715 °N, -93.098 °W; Waseca, 44.076 °N, -93.523 °W). Prior to planting, the fields were disked and leveled. The trellis was constructed with three 100 ft rows with 15 ft between each row and a trellis height of 16 ft. Two hop cultivars were selected for transplanting, cvs. Brewer's Gold (moderately resistant) and Columbus (susceptible) (Great Lakes Hops, Zeeland, MI). Plants were arranged with three replications using a split-split plot treatment design. Whole plots were designated as locations, subplots as cultivars, and sub-subplots as fungicide treatments. Replicated subplots (cultivars) were 50 ft in length and each replicated sub-subplot (fungicide treatment) contained two plants that were 3 ft apart with 4.5 ft between plots. The fields were hand-weeded as necessary. Nitrogen was applied at a rate of 160 lbs N/acre in both 2015 and 2016. Nitrogen was applied as three granular fertilizer applications and incorporated into the top 6 inches of soil. In Grand Rapids, nitrogen was applied as calcium nitrate (15.5-0-0 NPK). In Rosemount and Waseca, nitrogen was applied as urea (46-0-0 NPK).

In both 2015 and 2016, individual plants were trained on single strings of coir with 4 – 8 bines per plant. In 2015, single transplants of a downy and powdery mildew susceptible experimental breeding line were planted at both ends of each row at all locations to allow for inoculum spread. Prior to transplanting, these plants were

inoculated with a composite mixture of *P. humuli* sporangia derived from multiple basal spikes recovered from hopyards within Minnesota. Inoculum was prepared by rinsing heavily sporulating basal spikes with sterile distilled water and standardizing inoculum to 50,000 sporangia/mL. Plants were inoculated with this suspension and then placed into plastic bags for a period of 24 h following inoculation before being removed and transplanted into the field.

Field experiment 1. In 2015, scouting for initial signs of disease was initiated in mid-April at Rosemount and Waseca whereas scouting did not begin for Grand Rapids until mid-May due to differences in crop emergence. Fungicide applications began in May following emergence but prior to training of hop shoots with starting dates varying depending on location. Fungicide treatments (Table 2.1) consisted of a single, tank-mixed, or pre-mixed fungicide compounds applied at the highest rate allowable throughout the entire season and at recommended application intervals based on manufacturer instructions. Treatments included a non-treated control; extract of *Reynoutria sachlianensis* (Regalia®, Marrone Bio Innovations, Davis, CA) and copper hydroxide (Kocide® 3000, DuPont Crop Protection, Wilmington, DE); boscalid and pyraclostrobin (Pristine®, BASF Ag Products, Research Triangle Park, NC); copper hydroxide (Kocide® 3000, DuPont Crop Protection, Wilmington, DE); phosphorous acid (Agri-Fos®, AgriChem, Queensland, AU); or mefenoxam (Ridomil® Gold SL, Syngenta Crop Protection, Greensboro, NC) and copper hydroxide (Kocide® 3000, DuPont Crop Protection, Wilmington, DE). Fungicide applications and visual disease ratings were taken at 7 – 28 day intervals at all three locations. Disease ratings were assessed on a

whole plant basis using a 0–5 scale where 0 = no disease, 1 = 1–25% foliar disease, 2 = 26–50% foliar disease, 3 = 51–75% foliar disease, 4 = 76–100% foliar disease, 5 = dead plant. Visual disease ratings were averaged across two plants (sub-samples) within a plot for each replicate. By late July, fungicide applications ended due to lack of disease in all plots. Additional hop downy mildew inoculations were initiated in mid-August through early September to improve chances of disease in the following year.

Field experiment 2. In 2016, scouting for initial signs of disease was initiated in early April at Rosemount and Waseca whereas scouting did not initiate until early May in Grand Rapids due to differences in crop emergence. Fungicide applications did not begin until May following emergence but prior to training of hop shoots, with starting dates varying by location. Fungicide treatments (Table 2.2) consisted of a series of single, tank-mixed, or pre-mixed fungicides applied at varying rates throughout the season based on crop development with a fixed-interval schedule of 14 days between applications. Treatments included a non-treated control; a fungicide program that included extract of *Reynoutria sachlianensis* and copper hydroxide (Regalia®, Marrone Bio Innovations, Davis, CA, and Kocide® 3000, DuPont Crop Protection, Wilmington, DE), *Bacillus pumilis* Strain QST 2808 (Sonata®, Bayer CropScience, Research Triangle Park, NC), and *Streptomyces lydicus* WYEC 108 (Actinovate® AG, Novozymes BioAg, Franklinton, NC); a fungicide program that included phosphorous acid (Agri-Fos®, AgriChem, Queensland, AU), trifloxystrobin (Flint®, Bayer CropScience, Research Triangle Park, NC), mefenoxam and copper hydroxide (Ridomil® Gold SL, Syngenta Crop Protection, Greensboro, NC, and Kocide® 3000, DuPont Crop Protection, Wilmington, DE), and

mandipropamid (Revus®, Syngenta Crop Protection, Greensboro, NC); a fungicide program that included phosphorous acid (Agri-Fos®, AgriChem, Queensland, AU), boscalid and pyraclostrobin (Pristine®, BASF Ag Products, Research Triangle Park, NC), mefenoxam and copper hydroxide (Ridomil® Gold SL, Syngenta Crop Protection, Greensboro, NC, and Kocide® 3000, DuPont Crop Protection, Wilmington, DE), and mandipropamid (Revus®, Syngenta Crop Protection, Greensboro, NC); a fungicide program that included phosphorous acid (Agri-Fos®, AgriChem, Queensland, AU), cymoxanil and copper hydroxide (Curzate® 60DF and Kocide® 3000, DuPont Crop Protection, Wilmington, DE), mefenoxam and copper hydroxide (Ridomil® Gold SL, Syngenta Crop Protection, Greensboro, NC, and Kocide® 3000, DuPont Crop Protection, Wilmington, DE), and mandipropamid (Revus®, Syngenta Crop Protection, Greensboro, NC); or a fungicide program that included phosphorous acid (Agri-Fos®, AgriChem, Queensland, AU), cymoxanil, famoxadone, and copper hydroxide (Tanos® and Kocide® 3000, DuPont Crop Protection, Wilmington, DE), mefenoxam and copper hydroxide (Ridomil® Gold SL, Syngenta Crop Protection, Greensboro, NC, and Kocide® 3000, DuPont Crop Protection, Wilmington, DE), and mandipropamid (Revus®, Syngenta Crop Protection, Greensboro, NC). Visual disease ratings were taken bi-weekly throughout the growing season from mid-May until early September. Visual disease ratings were assessed on a whole plant basis using a 0–5 scale where 0 = no disease, 1 = 1–25% foliar disease, 2 = 26–50% foliar disease, 3 = 51–75% foliar disease, 4 = 76–100% foliar disease, 5 = dead plant. Disease ratings were averaged across two plants (sub-samples) within a plot for each replicate.

In vitro fungicide study. Fungicides registered for use in hop production were evaluated for control of hop downy mildew. Eleven different fungicides were evaluated including copper hydroxide (Kocide® 3000, DuPont Crop Protection, Wilmington, DE), cymoxanil (Curzate® 60DF, DuPont Crop Protection, Wilmington, DE), cymoxanil and famoxadone (Tanos®, DuPont Crop Protection, Wilmington, DE), extract of *Reynoutria sachalinensis* (Regalia®, Marrone Bio Innovations, Davis, CA), fluopicolide (Presidio®, Valent USA LLC Agricultural Products, Walnut Creek, CA), fosetyl-Al (Aliette® 50WDG, Bayer CropScience, Research Triangle Park, NC), mandipropamid (Revus®, Syngenta Crop Protection, Greensboro, NC), mefenoxam (Ridomil® Gold SL, Syngenta Crop Protection, Greensboro, NC), phosphorous acid (Agri-Fos®, AgriChem, Queensland, AU), pyraclostrobin and boscalid (Pristine®, BASF Ag Products, Research Triangle Park, NC), trifloxystrobin (Flint®, Bayer CropScience, Research Triangle Park, NC), and a water-treated control.

Three-week old rooted-cuttings of the susceptible cv. Pacific Gem were maintained in a greenhouse with a 16 h photoperiod, with temperatures ranging from 22.6 – 25.7 °C. Twenty-four hours before inoculation, fungicide treatments were applied to single rooted-cuttings at the highest recommended rate (Table 2.3) as a foliar application based on a total spray volume of 280 L/ha. Twenty-four hours after treatment, five healthy leaves were selected from three to five nodes below the apical meristem and placed individually with the abaxial surface facing upwards in a 90 mm Petri dish containing a single sterile paper towel wetted with 1.5 mL of sterile water. Inoculum was prepared on cv. Pacific Gem using detached leaf cultures in a similar manner. Sporangial suspensions were collected from heavily infected leaves by shaking them vigorously in a

50 mL Falcon tube with 30 mL of sterile water and adjusted to a concentration of 50,000 sporangia/mL. This inoculum originated as a composite mixture from isolates collected in Michigan, Minnesota, Oregon, and Wisconsin to reflect the diversity of plant material sources.

Approximately 1 mL of inoculum was applied to the fungicide treated leaves using a handheld spray bottle (US Plastics, Lima, OH) and the leaves were then placed in a growth chamber (Model #E15, Controlled Environments Ltd., Winnipeg, MB, Canada) for a period of seven days at 20 °C with a 14 h photoperiod. Seven days after inoculation (DPI) the leaves were removed from the growth chamber and images were collected using a CanoScan 110 LiDE scanner (Cannon USA, Melville, NY) using the default settings on a white background. Images were imported into ASSESS v2.0 (American Phytopathological Society, St. Paul, MN) and were evaluated for the total percent diseased leaf area using the default settings. This experiment was arranged in a randomized complete block design with ten replicates.

Statistical analysis. Data from the field experiment in 2015 were not analyzed due to inadequate disease incidence. Data from the field experiment conducted in 2016 were analyzed in a mixed effect model with a balanced dataset. Grand Rapids was removed from further analysis due to the lack of disease incidence. The experiment was analyzed as a split-split plot with locations, cultivars, and fungicides considered as fixed effects and replicates nested within locations were treated as a random effect. Locations were whole plot treatments, cultivars were subplot treatments, and fungicides were sub-subplot treatments. Due to issues with plant death over the course of the winter from an

unknown cause, replanting of damaged plots took place in early June of each year. Plant age was used as a covariate in further analyses to represent differences in disease incidence and severity. The area under the disease progress curve (AUDPC) value was calculated and used as the response variable to determine treatment effects. Mixed model analyses were conducted using JMP Pro 13 (SAS Institute, Cary, NC) following an evaluation of residual plots of the response variable. Mean separation procedures were performed using Tukey's HSD and Dunnett's test (α=0.05).

Data from the *in vitro* study were first evaluated to determine efficacy of fungicide treatments compared to a the mock-treated control and then following analysis the data were normalized to percent diseased leaf area compared to a mock-treated (H_2O) control. Both percent foliar disease and percent disease reduction were evaluated as the response variables. Fungicide treatment was considered a fixed effect and replicate was treated as a random effect. Mixed model analyses were conducted using JMP Pro 13 (SAS Institute, Cary, NC) following an evaluation of residual plots of the response variables. Additionally, means separation procedures were performed using Tukey's HSD and Dunnett's test (α=0.05).

Results and Discussion

Field experiment 1. In 2015 there were no results reported between treatments or locations, due to inadequate disease incidence and severity across locations. Establishing disease across locations is significantly influenced by weather factors and *P. humuli* zoospore infectivity is known to decrease significantly during a 24-hour period. While inoculum used for these assays was prepared fresh on the morning of the inoculations, we

cannot conclude whether the lack of infection was a result of spore viability or environmental factors.

Field experiment 2. In 2016 overall disease severity was significantly different between both locations (F = 12.337, P = 0.0246) with Waseca having moderate disease severity and Rosemount having high disease severity. Additionally there were significant main effects of fungicide treatment (F = 3.097, P = 0.0260) with the fungicide program containing Tanos (cymoxanil and famoxadone) controlling hop downy mildew significantly better when compared to the non-treated control (P = 0.0047). While this may be due to overall variability of disease incidence or severity within a field there is also concern about the effect of plant age on the incidence and severity of disease in a hopyard (Gent *et al*., 2012a). These results indicate that disease severity across locations is influenced to a great degree by the combination of cultural factors such as fungicide use and to a lesser extent, varietal selection. It may be that the disease scoring system we developed for these assays did not adequately differentiate the two cultivars used in this study (Woods and Gent, 2016). In areas where the environment is conducive to disease development, selection of resistant or tolerant host varieties in combination with effective fungicide treatment will be necessary to maintain adequate disease control (Johnson *et al*., 1983; Gent *et al*., 2010; Gent *et al*., 2012b). Also, in environments with limited disease incidence, use of resistant cultivars may be adequate to maintain effective disease control thereby reducing labor and additional input costs related to disease management although fungicide use is still recommended (Johnson *et al*., 1983; Johnson *et al*., 1991). Through consistent and timely fungicide applications along with host resistance and

additional cultural practices (e.g. pruning) that promote airflow and decrease humidity, hop producers can achieve adequate levels of disease control (Gent *et al*., 2012b).

In vitro fungicide study. All fungicides significantly reduced disease when compared to the mock-treated control ($F = 8.376$, $P = <0.0001$). Furthermore, following normalization to the mock-treated control, the total percent reduction in disease varied depending on the fungicide ($F = 1.969$, $P = 0.0459$). Interestingly, only fluopicolide performed significantly better than the biological fungicide, Regalia, as indicated by pairwise comparisons ($P = 0.0281$). Unfortunately, at the current time fluopicolide is not registered for use in commercial hop production, although its efficacy in the field has recently been demonstrated (Gent, 2017) though data from their study only represent a single year. Previous research has demonstrated that fungicide insensitivity to metalaxyl (and mefenoxam) and fosetyl-Al exists in certain hop production regions, which has major implications in newer production regions where registration of fewer fungicidal compounds is present for hop, thereby further limiting selection of effective controls (Gent *et al*., 2008; Hellwig *et al.*, 1991; Klein, 1994; Nelson *et al*., 2004). Additionally, insensitivity to fosetyl-Al may pose threats to other phosphonate fungicides which are commonly used in hop production for control of hop downy mildew. While these experiments demonstrated that mefenoxam and fosetyl-Al did increase disease control, even when mefenoxam was applied individually, it would be worthwhile to assay multiple pathogen isolates for sensitivity in newer production regions where these fungicides have seen limited use (Marks and Gevens, 2016). While these fungicides were evaluated in a controlled setting, field trials more naturally reflect the practical

aspect of on-farm production practices and future work should focus on both application intervals and production practices that influence early season disease severity in newer production regions. Lastly, validating epidemiological forecasting models used in traditional regions as a method of reducing input costs will contribute to the sustainability in newer production regions where timely fungicide applications are needed.

Conclusions

Fungicides are an integral component to crop production and provide a sound basis for reducing plant disease in commercial settings. Efficacy of fungicides will depend on numerous environmental factors and fungicide mechanisms of action. Additionally, varietal selection will play a key role in fungicide efficacy due to differences in susceptibility to pathogens in different regions. Demonstration of fungicide efficacy is a key factor for subsequent use, but caution should be exercised when sourcing plant materials. Due to the clonal nature of hop, potential introduction of fungicide insensitive strains of *P. humuli* is possible. Monitoring of transported plant materials to prevent such an occurrence is advisable, either through use of molecular diagnostics or bioassays. Additional work should be focused on fungicide sensitivity in Midwestern hop production and also to delineate population structure of *P. humuli* in the eastern United States. Since common hop is native to North America, there is evidence for native populations to act as a reservoir for *P. humuli* which may explain recent epidemics in the eastern United States. As demand for hop production increases in non-traditional regions, growers will also need to begin considering cost of production factors that influence their productivity while still maintaining the quality aspects that commercial

brewers are familiar with. Production costs are minimized when disease severity is low and yield per plant is high but varieties that are tolerant to the disease may be poorly-adapted to these newer regions and therefore costs are likely to be inflated as a result.

Table 2.1. Fungicide treatment programs for 2015 field season. Legend: Program = Fungicide treatment program; Trade Name = Common trade name of product; Active Ingredient = Chemical component with fungicidal activity; FRAC Code = Fungicide Resistance Action Committee (FRAC) code used to delineate similar mechanisms of action within chemical compound families; Rate (Units) = Rate of fungicidal compound used during an application period; Risk = Relative risk of pathogens to develop resistance to fungicide mechanism of action.

Program	Trade Name	Active Ingredient	FRAC Code	Rate (Units)	Risk
1	Non-treated	N/A	N/A	N/A	N/A
2	Kocide 3000*, Regalia**	copper hydroxide, plant extract	M, P5	1.5 lbs 4 qts	Low
3	Pristine†	boscalid + pyraclostrobin	7, 11	28 oz	Medium to High
4	Kocide 3000	copper hydroxide	M	1.5 lbs	Low
5	Agri-Fos††	phosphorous acid	33	3 qts	Low
6	Kocide 3000, Ridomil Gold SL‡	copper hydroxide, mefenoxam	M, 4	1.5 lbs 0.5 qts	Low to High

* = Manufactured by DuPont Crop Protection, Wilmington, DE

** = Manufactured by Marrone Bio Innovations, Davis, CA

† = Manufactured by BASF Ag Products, Research Triangle Park, NC

†† = Manufactured by Agri-Chem, Yatala, QLD, Australia

‡ = Manufactured by Syngenta Crop Protection, Greensboro, NC

Table 2.2. Fungicide treatment programs for 2016 field season. Legend: Program = Fungicide treatment program; Spray Order = Order in which fungicide treatments were applied; Trade Name = Common trade name of product; Active Ingredient = Chemical component with fungicidal activity; Rate (Units) = Rate of fungicidal compound used during an application period; Total # MOA = Total number of compounds with different a mechanism of action applied as a part of the fungicide program; FRAC Code = Fungicide Resistance Action Committee (FRAC) code used to delineate similar mechanisms of action within chemical compound families; Risk = Relative risk of pathogens to develop resistance to fungicide mechanism of action; Total Field Use EIQ = Total Field Use Environmental Impact Quotient (EIQ) for the specific fungicide program; Total Cost ($)/ Acre = Total cost of fungicide program per acre based on usage rate; Cost ($)/Pound (lb) of Dried Hops = Total cost of fungicide program per pound of dried hops produced (assuming dry yield of 1500 pounds per acre).

Program	Spray Order	Trade Name	Active Ingredient	Rate (Units)	Total # MOA	FRAC Code	Risk	Total Field Use EIQ	Total Cost ($)/ Acre	Cost ($)/Pound (lb) of Dried Hops
1	NA	Non-treated control	NA	N/A	0	0	NA	0	0.00	0.00
2	1, 3, 5, 7, 8	Kocide 3000*, Regalia**	copper hydroxide, plant extract	1.5 lbs and 2, 2, 4, 4, 4 qts	4	M, P5	Low	115.5	193.61	0.13
	2, 6	Sonata***	*Bacillus pumilis* Strain QST 2808	3.2, 7 qts		M	Low			
	4	Actinovate AG\\	*Streptomyces lydicus* WYEC 108	10 oz		M	Low			
3	1, 4, 6	Agri-Fos††	phosphorous acid	1.5, 3.9, 5.25 fl oz	5	33	Low	125.4	249.67	0.17
	2, 5, 7	Flint***	trifloxystrobin	3, 4, 4 oz		11	High			
	3	Kocide 3000, Ridomil Gold SL‡	copper hydroxide, mefenoxam	1.5 lbs and 0.5 pts		M, 4	Low to High			
	8	Revus‡	mandipropamid	8 fl oz		40	Low to Medium			
4	1, 4, 6	Agri-Fos	phosphorous acid	1.5, 3.9, 5.25 fl oz	5	33	Low	152.0	311.42	0.21
	2, 5, 7	Pristine†	boscalid + pyraclostrobin	14, 21, 28 oz		7, 11	Medium to High			
	3	Kocide 3000, Ridomil Gold SL	copper hydroxide, mefenoxam	1.5 lbs and 0.5 pts		M, 4	Low to High			
	8	Revus	mandipropamid	8 fl oz		40	Low to Medium			
5	1, 4, 6	Agri-Fos	phosphorous acid	1.5, 3.9, 5.25 fl oz	5	33	Low	197.4	187.67	0.13
	2, 5, 7	Kocide 3000, Curzate*	copper hydroxide, cymoxanil	1.5 lbs and 3.2 oz		M, 27	Low to Medium			
	3	Kocide 3000, Ridomil Gold SL	copper hydroxide, mefenoxam	1.5 lbs and 0.5 pts		M, 4	Low to High			
	8	Revus	mandipropamid	8 fl oz		40	Low to Medium			
6	1, 4, 6	Agri-Fos	phosphorous acid	1.5, 3.9, 5.25 fl oz	5	33	Low	201.6	223.67	0.15
	2, 5, 7	Kocide 3000, Tanos*	copper hydroxide, cymoxanil + famoxadone	1.5 lbs and 8 oz		M, 11, 27	Low to High			
	3	Kocide 3000, Ridomil Gold SL	copper hydroxide, mefenoxam	1.5 lbs and 0.5 pts		M, 4	Low to High			
	8	Revus	mandipropamid	8 fl oz		40	Low to Medium			

* = Manufactured by DuPont Crop Protection, Wilmington, DE

** = Manufactured by Marrone Bio Innovations, Davis, CA

*** = Manufactured by Bayer Crop Science, Research Triangle Park, NC

\\ = Manufactured by Novozymes BioAg, Franklinton, NC

† = Manufactured by BASF Ag Products, Research Triangle Park, NC

†† = Manufactured by Agri-Chem, Yatala, QLD, Australia

‡ = Manufactured by Syngenta Crop Protection, Greensboro, NC

Table 2.3. Fungicide treatments evaluated for *in vitro* fungicide study. Legend: Trade Name = Common trade name of product; Active Ingredient = Chemical component with fungicidal activity; Rate (Units) = Recommended rate of fungicidal compound used during an application period; FRAC Code = Fungicide Resistance Action Committee (FRAC) code used to delineate similar mechanisms of action within chemical compound families; Risk = Relative risk of pathogens to develop resistance to fungicide mechanism of action.

Trade Name	Active Ingredient	Rate (Units)	FRAC Code	Risk
Water Control (H2O)	N/A	N/A	N/A	N/A
Agri-Fos††	phosphorous acid	0.975 qts	33	Low
Aliette 50 WDG***	fosetyl-Al	2.5 lbs	33	Low
Curzate*	cymoxanil	3.2 oz	27	Low to medium
Flint***	trifloxystrobin	1 oz	11	High
Kocide 3000*	copper hydroxide	1.5 lbs	M	Low
Presidio//	fluopicolide	4 fl oz	43	Unknown
Pristine†	boscalid + pyraclostrobin	4.2 oz	7 + 11	Medium to high
Regalia**	plant extract	1 qt	M	Low
Revus‡	mandipropamid	8 fl oz	40	Low to medium
Ridomil Gold SL‡	mefenoxam	0.5 pts	4	High
Tanos*	cymoxanil + famoxadone	8 oz	11 + 27	Low to high

* = Manufactured by DuPont Crop Protection, Wilmington, DE

** = Manufactured by Marrone Bio Innovations, Davis, CA

*** = Manufactured by Bayer Crop Science, Research Triangle Park, NC

// = Manufactured by Valent USA LLC Agricultural Products, Walnut Creek, CA

† = Manufactured by BASF Ag Products, Research Triangle Park, NC

†† = Manufactured by Agri-Chem, Yatala, QLD, Australia

‡ = Manufactured by Syngenta Crop Protection, Greensboro, NC

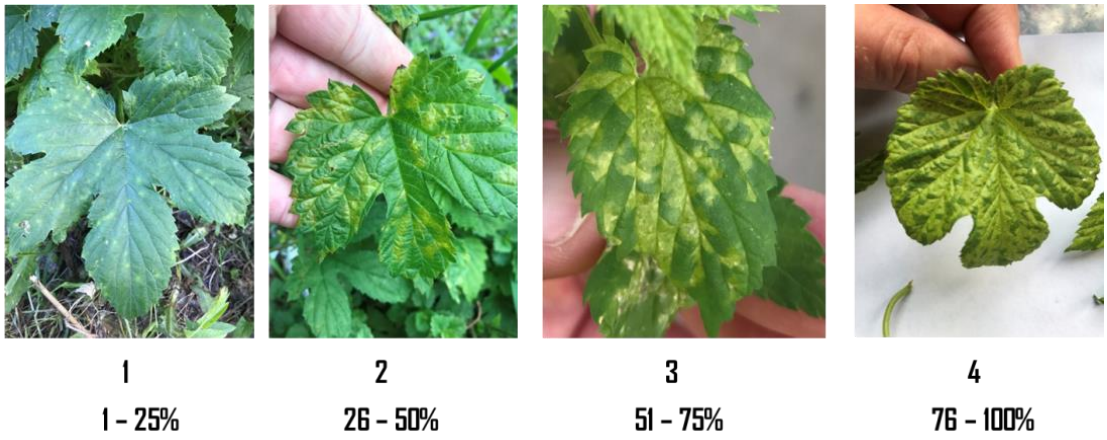

Figure 2.1. Hop downy mildew disease rating scale used for fungicide evaluations. 0 = no disease, 1 = 1–25% foliar disease, 2 = 26–50% foliar disease, 3 = 51–75% foliar disease, 4 = 76–100% foliar disease, 5 = dead plant.

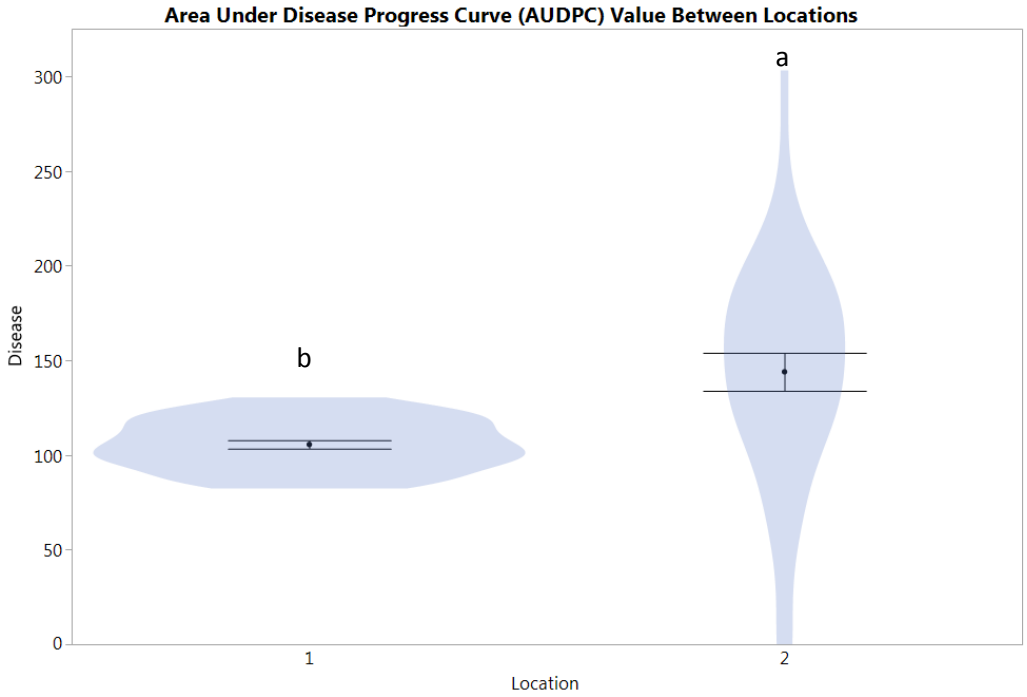

Figure 2.2. Violin plot of area under the disease progress curve (AUDPC) values between locations in 2016 field trial. Legend: Location 1 = Waseca, Location 2 = Rosemount. Mean and standard error are displayed (n = 3). Letters above SE bars indicate significance groupings as determined by Tukey's HSD; bars with the same letter do not differ significantly from one another (F = 12.337, P = 0.0246).

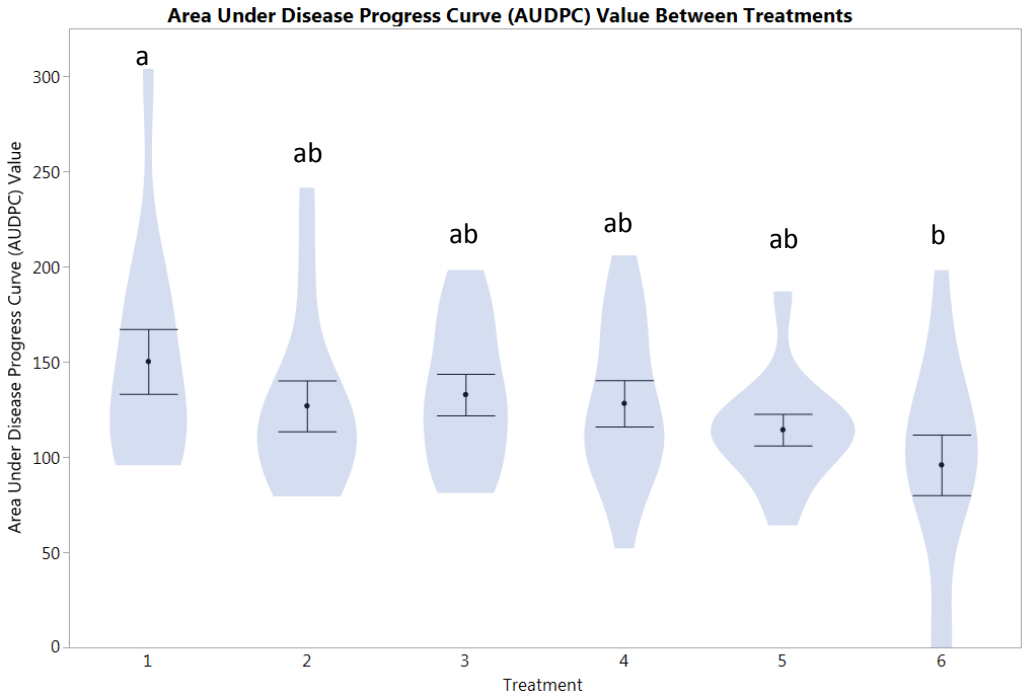

Figure 2.3. Violin plot of area under disease progress curve (AUDPC) value between fungicide treatments in 2016 field trial. Legend = Each treatment corresponds to its specific fungicide program (i.e. treatment 1 = non-treated control). Mean and standard error are displayed (n = 3). Letters above SE bars indicate significance groupings as determined by Dunnett's test; bars with the same letter do not differ significantly from one another (F = 3.097, P = 0.0260).

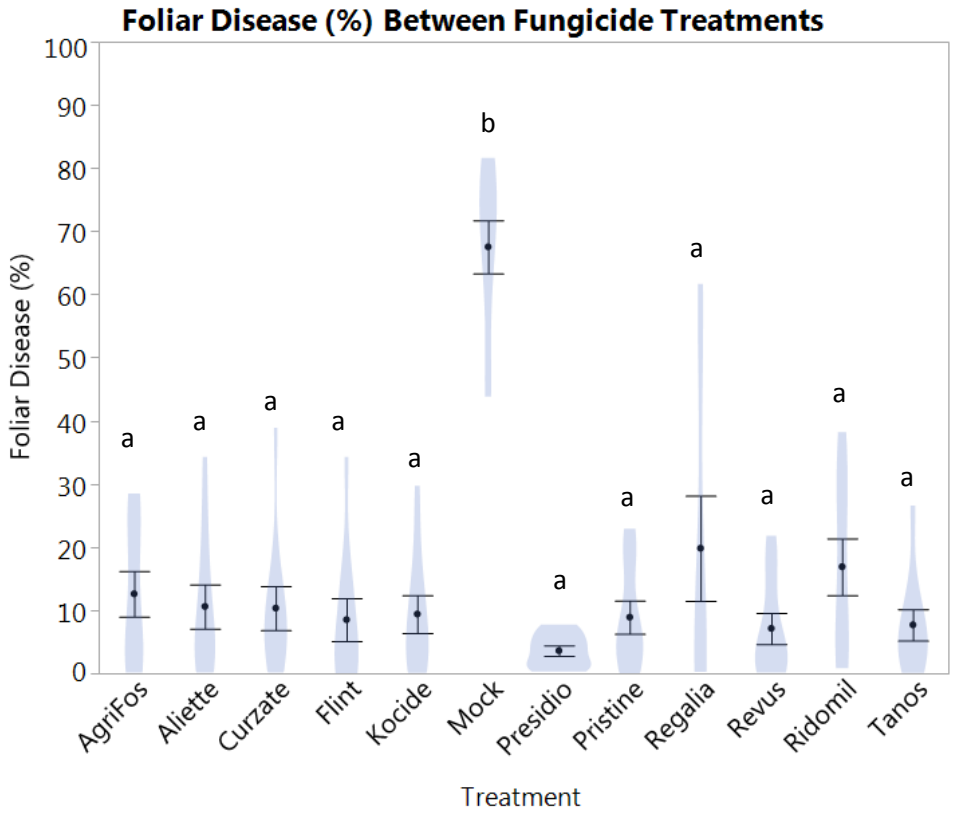

Figure 2.4. Violin plot of percent foliar disease following fungicide treatments using an *in vitro* assay. A single replicate of five leaves from each plant was collected in the early morning and each leaf was placed abaxial surface up onto a sterile, moist paper towel inside of a 90 mm Petri dish. The abaxial leaf surface was misted using a handheld atomizer with approximately 1 mL (50,000 sporangia) of prepared P. humuli inoculum. Plates were placed in a growth chamber at 20 °C with a 14 h photoperiod and incubated for 7 days following inoculation. The leaves were then digitally scanned using a Cannon LiDE 1100 flatbed scanner, images were uploaded into APS ASSESS v2.0 and the percent diseased leaf area was determined using standard settings. Mean and standard error (SE) are displayed (n = 10). Letters above SE bars indicate significance groupings as determined by Dunnett's test; bars with the same letter do not differ significantly from one another (F = 8.376, P = <0.0001).

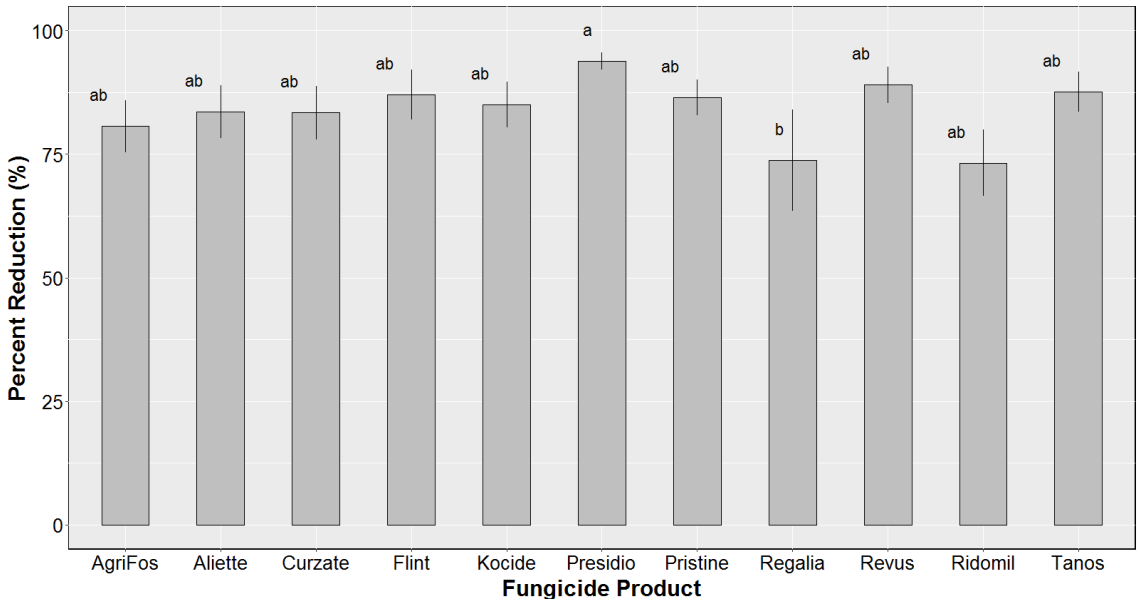

Figure 2.5. Percent reduction in foliar disease amount compared to mock-treated (water) control. A single replicate of five leaves from each plant was collected in the early morning and each leaf was placed abaxial surface up onto a sterile, moist paper towel inside of a 90 mm Petri dish. The abaxial leaf surface was misted using a handheld atomizer with approximately 1 mL (50,000 sporangia) of prepared P. humuli inoculum. Plates were placed in a growth chamber at 20 °C with a 14 h photoperiod and incubated for 7 days following inoculation. The leaves were then digitally scanned using a Cannon LiDE 1100 flatbed scanner, images were uploaded into APS ASSESS v2.0 and the percent diseased leaf area was determined using standard settings. Mean and standard error (SE) are displayed (n = 10). Letters above SE bars indicate significance groupings as determined by Tukey's HSD; bars with the same letter do not differ significantly from one another (F = 1.969, P = 0.0459).

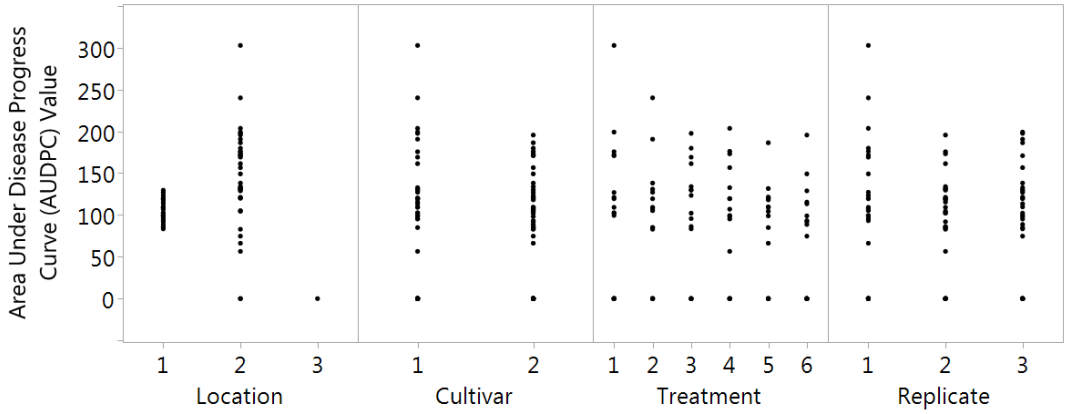

Figure 2.6. Scatterplot matrix of area under the disease progress curve (AUDPC) value, grouped by location, cultivar, fungicide treatment, and replicate. Locations = Waseca (1), Rosemount (2), and Grand Rapids (3). Cultivar = Brewer's Gold (1), Columbus (2). Treatment = Each number represents the corresponding fungicide program evaluation in 2016.

Chapter 3

Phenotypic Characterization of Wild North American Hop (*Humulus lupulus* L.) for Foliar Resistance to Hop Downy Mildew (*Pseudoperonospora humuli* Miy. et. Takah.)

Summary

Pseudoperonospora humuli Miy. et. Takah., the causal organism of hop downy mildew, is a devastating oomycete pathogen of common hop (*Humulus lupulus* L.). Resistance to hop downy mildew comes from a limited number of sources, primarily of European descent. Genetic control of resistance to hop downy mildew is thought to be highly-quantitative. Here we report the collection of novel hop germplasm resources from the Midwestern United States and subsequent evaluations of hop downy mildew resistance of wild North American and Eurasian hop accessions via detached-leaf assays and whole plant inoculations. In total, 17 collection sites in the Midwestern United States were visited and yielded 72 clonal accessions and approximately 26,000 seed. Initial screening of different species and taxonomic varieties of hop indicated that resistance could likely be identified in material native to the north central United States (*H. lupulus* var. *lupuloides*), as well as the related annual species *H. japonicus*. Additional screening of 112 wild hop accessions from various germplasm collections using a detached leaf assay indicated that significant differences exist between regions of wild hops origin, with hops originating from the United States possessing greater levels of foliar resistance than those from Canada.

Introduction

Common hop (*Humulus lupulus*) is a twining, dioecious, perennial plant. The hop plant is cultivated commercially for the female inflorescences which contribute to bitterness in beer (Neve, 1991). Hop species are native to the northern hemisphere, with its natural range encompassing Eurasia and North America. Hop downy mildew is caused by the homothallic oomycete pathogen *Pseudoperonospora humuli* (Miy. et Tak.) Wils. and is considered one of the most economically damaging diseases of hop (Gent *et al*., 2017; Johnson *et al.*, 2009; Royle and Kremheller, 1981). The disease is considered ubiquitous in commercial production in the northern hemisphere, especially in cool, wet climates of the continental United States. Resistance to the disease is thought to derive from a limited number of founder plants originating from Europe (Woods and Gent, 2016). Due to the devastating nature of the disease, it is necessary to continually improve commercial varieties of hop by identifying and selecting genetically diverse sources of resistance to *P. humuli*.

The center of origin of hop is in western China and common hop can be demarcated into five taxonomic varieties (var. *cordifolius*, var. *lupuloides*, var. *lupulus*, var. *neomexicanus*, and var. *pubescens*) based on floral leaf morphological characters (Small, 1978). Three of the five taxonomic varieties are native to North America (var. *lupuloides*, var. *neomexicanus*, and var. *pubescens*) while the remaining two (var. *cordifolius* and var. *lupulus*) are native to Eurasia and Japan (Murakami, 2006; Neve, 1991; Small, 1978). In North America, hop inflorescences ("cones") were initially collected from the wild for use in brewing and subsequent introduction of European cultivars led to naturalized populations of *H. lupulus* var. *lupulus* in the northeastern

United States (Small, 1978; Tomlan, 2013). Historically, breeding programs focused on clonal evaluation of landrace cultivars but improved cultivars were primarily the result of hybridization between native North American and European hops (Darby, 2006; Salmon, 1934). Cultivars derived from these crosses are ancestors of most of the currently grown cultivars worldwide (Darby, 2006; Woods and Gent, 2016).

Spring emergence of shoots systemically infected with *P. humuli*, termed "basal spikes", provide primary inoculum (Coley-Smith, 1964; Skotland, 1961) that subsequently initiates new infections that often occur as aggregated clusters of infected plants throughout a hopyard (Gent *et al.*, 2012b; Johnson *et al.*, 1991). If the resulting disease is left unmanaged, secondary foliar infections can lead to the infection of lateral branches, which bear the inflorescences, causing a reduction in cone yield and quality. Additionally, hop downy mildew can become a persistent and perennial issue in newly-established hopyards especially when sourcing and phytosanitary certification of plants is not considered prior to planting (Turner *et al*., 2011). Plants that are especially susceptible can die as a result of the systemic infection within one or a few seasons (Woods and Gent, 2016).

Recently, wild North American hops were recovered from the western United States in an effort to identify resistance to hop powdery mildew (Smith, 2005). Smith (2005) identified *H. l.* var. *lupuloides* from North Dakota, Manitoba, and Saskatchewan as containing the highest frequency of powdery mildew resistant or tolerant genotypes. To date, only a limited number of studies focused on the use of wilds hops as sources of novel disease resistance have been conducted (Seigner *et al*., 2005). It was our objective

to sample from local, native populations of hop and to screen wild germplasm resources for foliar resistance to *P. humuli*.

Materials and Methods

Hop germplasm collection. Locale data were aggregated from the USDA Germplasm Resources Information Network (GRIN) and the University of Minnesota Bell Museum of Natural History, St. Paul, MN (MN). We also collected local landowner observations since certain collections occurred on private property. Seed lots from GRIN were selected to randomly survey hop populations across the known range and included sampling of all three native botanical varieties, a single non-native botanical variety (var. *lupulus*), and the non-native annual species, *H. japonicus* (Table 3.1). Seeds were surface-sterilized with a solution of 20% bleach for 15 minutes and rinsed with sterile distilled water three times before being placed into 90 mm Petri dishes containing sterile moistened sand. Seeds were stratified at 4 °C for a period of 8 weeks prior to placement in a growth chamber for germination at 20 °C under a 12 h photoperiod. Germinated seedlings were then transplanted into LC8 potting media (SunGro Horticulture, Bellevue, WA) in 50-cell flats and allowed to grow for a period of 3 weeks in a greenhouse under a 16 h photoperiod at 22 °C (± 3 °C) before being transplanted into 1 gal pots. Locations identified via herbaria and landowner observations were visited once in fall (September - December) in 2015 or spring in 2016. Rhizomes were collected from mature plants and if present, inflorescences containing fruits from female plants were sampled to obtain seeds. Rhizomes were washed free from contaminating soil and transplanted into a 1 gal container with LC8 potting media and maintained in a greenhouse devoid of *P. humuli*

under a 16 h photoperiod at 22 °C (± 3 °C) and fertilized once weekly with a solution of 400 ppm N (Peters 20-10-20 NPK, J. R. Peters, Allentown, PA) and irrigated as needed. Inflorescence material was dried, macerated, and seeds were cleaned of any contaminating debris.

Hop botanical variety and germplasm detached-leaf screening. *P. humuli* inoculum, originated as a composite mixture from isolates collected in Michigan, Minnesota, Oregon, and Wisconsin, was maintained on detached leaves of the susceptible cv. Pacific Gem. For experimental purposes, *P. humuli* inoculum comprised of sporangial suspensions was prepared by collecting heavily infected leaves of the downy mildew-susceptible cv. Pacific Gem and shaking vigorously in a 50 mL Falcon tube with 30 mL of sterile water. Inoculum concentration was estimated and standardized to 50,000 spores/mL with the aid of a hemocytometer.

In an initial set of experiments, we randomly-selected a single genotype from each of three hop botanical varieties, *H. l.* var. *lupuloides*, var. *lupulus*, and var. *neomexicanus*, and the related annual species Japanese hops (*H. japonicus*) for resistance screening (Table 3.3). A single replicate of five leaves from each plant was collected in the early morning and each leaf was placed individually abaxial surface up onto a sterile paper towel inside of a 90 mm Petri dish wetted with 1.5 mL sterile water. The abaxial leaf surface was misted using a handheld spray bottle with approximately 1 mL of inoculum (US Plastics, Lima, OH). Plates were then placed in a growth chamber (Model #E15, Controlled Environments Ltd., Winnipeg, MB, Canada) at 20 °C with a 14 h photoperiod and incubated for seven days post inoculation (DPI). The leaves were then

digitally scanned using a Cannon LiDE 1100 flatbed scanner (Cannon USA, Melville, NY) using default settings on a white background. Images were uploaded into APS ASSESS v2.0 (American Phytopathological Society, St. Paul, MN) and the percent diseased leaf area was determined using standard settings. This experiment was arranged in a randomized-complete block design and repeated six times.

Based on our preliminary results a subsequent experiment was carried out on 112 randomly selected genotypes (Table 3.4) that were sampled from the same germplasm collection. A single replicate of five leaves from each plant was collected as previously described and screened in an identical manner. This experiment was repeated eight times.

Whole plant screening. The abaxial leaf surfaces of three-week old rooted-cuttings of six selected accessions ('1006.02', cv. Centennial, 'Hohnke', cv. Pacific Gem, cv. Teamaker, and 'Waldenheimer') were inoculated using inoculum prepared as described above by lightly misting the abaxial surfaces of the leaves and incubating for 24 h at 20 °C after placing the whole plant in a plastic bag out of direct sunlight in a greenhouse. Following the incubation period, plants were removed from the plastic bags and placed into a greenhouse devoid of *P. humuli* with a 16 h photoperiod at 22 °C (± 3 °C) and fertilized once weekly with a solution of 400 ppm N (Peters 20-10-20, J. R. Peters, Allentown, PA) and irrigated as needed. Two weeks following inoculation, five leaves were randomly-selected from each of three plants (replicates) and digitally-scanned using the previously described methods. This experiment was repeated six times.

Correlation analysis of the hop downy mildew phenotype. We collected trait mean data from previously published studies reporting the percentage of foliar disease and proportion of systemically-infected shoots or basal spikes (Kralj *et al*., 1998; Woods and Gent, 2016). Briefly, Woods and Gent (2016) assessed 110 hop cultivars for their proportion of infected shoots from 2005 to 2007 in an unsprayed hopyard that was chemically pruned in both 2006 and 2007 for horticultural reasons. Assessments were made on 14 day intervals over three or four assessment periods during each year. Kralj *et al*. (1998) assessed 100 hop cultivars and breeding lines in an unsprayed hopyard. Assessments were made twice, during May and June, on leaves up to one meter of height on ten plants and foliar severity was estimated and the degree of infection was calculated. We selected common genotypes from each dataset (N = 44, Table 3.5) and conducted a correlation analysis of the combined dataset with the goal of identifying predictive relationships between resistance phenotypes to use in subsequent evaluations of germplasm.

Statistical analysis. Data from our three screening experiments were analyzed independently as mixed effects models as a randomized complete block design. In the first experiment in which we assessed the differences between taxa, taxon was considered a fixed effect and replicate as a random effect. In the second experiment in which we evaluated a larger germplasm collection, country of origin was considered a fixed effect and replicate and genotype nested within country of origin as a random effect. In our third experiment in which we assessed a subset of whole plants, accession was considered a fixed effect and replicate was considered a random effect. In all three experiments,

percent foliar disease was the response variable and was log-transformed for subsequent analyses. All mixed effects models and correlation analyses were performed using JMP Pro 13 (SAS Institute Inc., Cary, NC). Additionally, means separation was performed using Tukey's HSD (α=0.05).

Results and Discussion

Hop germplasm collection. In total, 16 sites in Minnesota and one site in Michigan were visited during 2015. Of these, one site contained *H. japonicus* and seeds were collected from multiple individuals and bulked together. Of the 16 other sites visited 15 contained previously unreported populations of hop (Table 3.2, Figure 3.1). As described previously (Hampton *et al.*, 2001; Smith *et al.*, 2006), it was common (6 sites, 37.5%) to encounter a solitary specimen which was unpollinated or of undetermined sex. In total, the 17 sampled sites yielded 17 seed and 72 plant accessions. From the USDA GRIN collection (Table 3.1, Figure 3.1), only 43 of 65 seed lots germinated. Of the 43 seed lots that germinated, there was an average germination rate of 19.0% and total seed germination ranged from 1 to 16 seeds per lot (Table 3.1).

Hop botanical variety and germplasm detached-leaf screening. Our initial characterization of the hop downy mildew resistant phenotype based upon experimental trials of interspecific groups of hop support previous observations made by Hoerner (1940) and Mancino (2013) in which limited, if any, disease develops on the annual species *H. japonicus*. Our results further indicate that there are significant differences (F= 22.567, *P*= <0.0001) within the sub-taxonomic groups of hop, with *H. lupulus* var.

lupuloides potentially being a source of novel resistance to *P. humuli*, though we cannot conclude if this effect was due to the specific genotype being evaluated since only one accession was chosen to represent each taxon (Figure 3.2).

To further test this hypothesis, we evaluated an expanded set of germplasm from diverse locations (Table 3.1, Table 3.2, and Table 3.3) which represented four of the five sub-taxonomic groups in *Humulus* spp., as well as accessions of uncharacterized sub-taxonomic status. Statistical analysis of percent foliar disease from 112 accessions indicated there were significant differences (F= 3.4989, *P*= 0.0337) in percent foliar disease, with accessions originating from Canada being more highly-susceptible than those originating from the United States (Figure 3.3 and 3.4). Since inoculum used in these assays originated from regions where commercial production occurs in the United States, we cannot conclude if inoculum originating from Canada or Eurasia would give different results though others have suggested this may not play a significant role (Gent *et al.*, 2017; Summers *et al*., 2015). Previous results have indicated that material from North America may contain limited sources of resistance but these reports have primarily been biased towards breeding varieties that contain significant population structure due to historical introgression events (Woods and Gent, 2016). The introgression of native North American hops with European hops is typified by the wild Manitoban hop, 'BB1', and several *H. lupulus* var. *neomexicanus* accessions, which was the primary foundation of the English breeding program (Darby, 2006). Given the differences of sub-taxonomic status and country of origin in resistance, we suggest the importance of distinguishing accessions of *H. lupulus* var. *lupuloides* recovered from the north central United States, specifically Minnesota and North Dakota for use in breeding hops for Minnesota and

proximal production areas, as opposed to southern regions in the Canadian provinces, Saskatchewan and Manitoba, where hop has been collected from. These accessions possessed higher levels of foliar resistance to *P. humuli* when compared to their more northern (or southern) counterparts.

Whole plant screening. There were significant differences (F= 13.659, *P*= <0.0001) amongst the six accessions evaluated for whole plant response to hop downy mildew infection. The two wild accessions from Michigan, 'Hohnke' and 'Waldenheimer', were as susceptible as cv. Pacific Gem, which displayed moderate susceptibility. Henning *et al.* (2016) report cv. Teamaker to be highly resistant to systemic ("crown") infection but in this study it performed similarly to moderately susceptible accessions in terms of foliar resistance. The wild accession '1006.02' displayed intermediate levels of foliar resistance, comparable to that of the downy-mildew tolerant genotype cv. Centennial (Kenny and Zimmermann, 1991), although it was not significantly different from cv. Teamaker or the wild Michigan accession 'Waldenheimer' (Figure 3.5). Differences in disease resistance across genotypes may be more pronounced under field conditions, where inocula are likely to be more spatially variable and prone to environmental influences compared to the pathogen favorable conditions created with the controlled environment and standardized inoculum levels deployed in our experiments.

Comparison across these studies is complicated by the fact that foliar assays with hop downy mildew are commonly conducted using a subjective ordinal scale (Henning *et al.*, 2015; 2016) whereas we evaluated foliar severity as an objective quantitative measurement (percent foliar disease) using a digital image analysis tool (APS ASSESS).

Our results might have benefitted from subsequent evaluation of additional disease phenotypes such as determining the proportion of infected shoots as performed by Woods and Gent (2016). However, this extends the length of time necessary to perform evaluations and detracts from developing a high-throughput method for evaluation of large breeding populations, common in most breeding programs. An additional method commonly employed, though not part of this study, is to inoculate small seedlings and score resistance based upon percentage of seedlings that develop terminal shoot infections. This provides family or population level information about the nature of resistance in any given cross and is considered a main method for evaluation of male breeding lines (Darby, 2005; Hoerner, 1932). This assay may have proved useful for evaluation of seedling plants but difficulty in recovering seed from certain locations didn't provide enough seedlings to allow such assays to take place. An additional concern is that a systemic infection arises following foliar inoculation thus confounding potential differences in the observed phenotypes.

Correlation analysis of hop downy mildew resistance phenotypes. Previous studies have evaluated resistance to hop downy mildew in the field, but consistency across locations and research groups is often problematic due to differences in disease scoring or experimental methods, which may include controlled inoculations or reliance upon natural infestations. A recent study by Woods and Gent (2016) described associations with region of origin of hop and disease resistance, but that study evaluated a collection of related cultivars sharing the historical introgression of a wild Manitoban hop referred to as 'BB1' and other founding cultivars. Additionally, a number of studies evaluating

genetic or metabolic markers associated with resistance to downy mildew describe the likelihood of numerous genetic loci which influence the downy mildew phenotype (Henning *et al*., 2015; 2016; Kralj *et al*., 1998; Parker, 2007). Henning *et al*. (2016) also evaluated multiple disease phenotypes (basal spikes vs. foliar lesions) of a bi-parental population within different environments (field vs. greenhouse) which led to detection of different genetic loci. Henning *et al.* (2016) demonstrated correlation (r = 0.54-0.57) between their greenhouse and field screenings but resistance phenotypes varied among environments. Results of our analysis of data from two independent studies (Kralj *et al*, 1998; Woods and Gent, 2016) evaluating two different downy mildew phenotypes suggest there is a significant linear relationship (r = 0.57, P = <0.001) between levels of foliar and crown resistance, which supports claims made by Henning *et al.* (2016) that the two phenotypes are correlated (Figure 3.6). Inconsistencies in resistance phenotypes amongst hop accessions may be related to differences in pathogen isolates though this is unlikely as Summers *et al*. (2015) recently demonstrated the lack of genetic diversity present in *P. humuli* and there is no currently published research to suggest differences in virulence among different isolates. Owing to the similarities between our correlation analysis and those conducted previously, it would seem that the phenotypes are relatively stable across time with environments contributing a larger role to disease manifestation and development due to differences in inoculum levels or climatological factors (Johnson *et al.*, 1983; Woods and Gent, 2016). Alternatively, a more important factor that may influence the outcome of our interpretation is the relative vigor or performance of a given hop accession in a given environment acting as a potential source of variation that might contribute to inconsistencies in observed resistance phenotypes.

Conclusions

The rapid expansion of the hop industry into non-traditional regions will require the utilization and development of locally-adapted genotypes that possess high levels of resistance to hop downy mildew. Our results document that hops growing natively in regions conducive to disease development, such as the Midwestern United States, may have value as a resource for novel disease resistance for improvement of cultivated hop. Additionally, correlation analysis of publicly available data further supports the idea that the two common disease phenotypes (percent foliar disease and proportion of systemically infected shoots) used for evaluating resistance to hop downy mildew (Kralj *et al*.,1998; Woods and Gent, 2016) are correlated with each other and either can likely be used as a primary indicator during gene discovery or early selection in breeding programs to reduce the cost and size of plantings for further trait evaluations. Due to the nature of the resistance phenotypes, further work is needed to determine whether the two phenotypes are controlled by distinct genetic loci or if they are a result of the same loci. Lastly, work is currently underway to evaluate the genetic diversity of the germplasm collection using next-generation sequencing technologies.

Table 3.1. Passport data of hop (*Humulus* spp.) seed accessions from USDA Germplasm Resources Information Network (GRIN) evaluated and germination rates.

Plant Introduction #	# Germinated	# Seed	Taxon	Country	State
559273	0	25	japonicus	United States	Kentucky
635242	5	25	lupuloides	United States	North Dakota
635243	10	25	lupuloides	United States	North Dakota
635244	7	24	lupuloides	United States	North Dakota
635246	3	25	lupuloides	United States	North Dakota
635247	3	26	lupuloides	United States	North Dakota
635251	2	25	lupuloides	Canada	Saskatchewan
635252	6	25	lupuloides	Canada	Saskatchewan
635254	0	10	lupuloides	Canada	Saskatchewan
617471	3	25	pubescens	United States	Missouri
617472	0	10	japonicus	United States	Nebraska
617473	9	25	japonicus		
635261	1	25	lupulus	Kazakhstan	
635262	1	25	lupulus	Kazakhstan	
635279	4	25	lupuloides	Canada	Manitoba
635285	4	25	lupuloides	United States	North Dakota
635288	9	26	lupuloides	United States	North Dakota
635289	0	25	lupuloides	Canada	Saskatchewan
635294	0	25	lupuloides	Canada	Saskatchewan
635298	0	25	lupuloides	Canada	Saskatchewan
635300	0	25	lupuloides	Canada	Saskatchewan
635302	4	25	lupuloides	Canada	Saskatchewan
635304	2	25	lupuloides	Canada	Saskatchewan
635305	3	25	lupuloides	Canada	Saskatchewan
635309	2	26	lupuloides	Canada	Saskatchewan
635312	4	25	lupuloides	Canada	Saskatchewan
635317	7	25	lupuloides	Canada	Saskatchewan
635322	1	25	lupuloides	Canada	Manitoba
635331	0	25	lupuloides	Canada	Saskatchewan

635337	0	20	lupuloides	Canada	Saskatchewan
635340	0	10	lupuloides	Canada	Manitoba
635344	0	25	lupuloides	Canada	Manitoba
634346	0	25	lupuloides	Canada	Manitoba
635351	6	25	lupuloides	Canada	Saskatchewan
635359	1	25	lupuloides	Canada	Saskatchewan
635362	2	25	lupuloides	Canada	Saskatchewan
635365	3	25	lupuloides	Canada	Saskatchewan
635368	5	23	lupuloides	Canada	Saskatchewan
635371	3	25	lupuloides	Canada	Saskatchewan
635379	10	25	lupuloides	Canada	Saskatchewan
635390	3	25	lupuloides	Canada	Saskatchewan
635402	1	25	lupuloides	Canada	Saskatchewan
635418	6	25	lupuloides	Canada	Saskatchewan
635431	8	24	neomexicanus	United States	Colorado
635433	12	25	neomexicanus	United States	Colorado
635435	0	25	neomexicanus	United States	Colorado
635437	1	25	neomexicanus	United States	Colorado
635441	0	25	neomexicanus	United States	Colorado
635444	0	25	neomexicanus	United States	Colorado
635446	3	25	neomexicanus	United States	Colorado
635453	0	27	neomexicanus	United States	Colorado
635455	0	25	neomexicanus	United States	Colorado
635458	1	24	neomexicanus	United States	Colorado
635463	1	25	neomexicanus	United States	Colorado
635464	1	25	neomexicanus	United States	Colorado
635467	0	25	neomexicanus	United States	Colorado
635477	13	24	neomexicanus	United States	New Mexico
635482	1	25	neomexicanus	United States	New Mexico
635484	0	25	neomexicanus	United States	New Mexico
635486	2	25	neomexicanus	United States	Colorado
635492	0	24	lupuloides	United States	North Dakota
635226	16	25	neomexicanus	United States	Arizona

Table 3.2. Location data of hop (*Humulus* spp.) accessions collected from Minnesota and Michigan during 2015 and 2016.

County	Clones Collected	Seed Collected
Anoka	3	0
Blue Earth	5	214
Blue Earth	1	0
Clearwater	1	272
Fillmore	1	0
Fillmore	4	0
Hennepin	4	0
Hennepin	27	24,609
Hennepin	2	11
Houston	8	24
Itasca	5	0
Le Seuer	1	0
St. Louis	0	349
St. Louis	1	627
Winona	1	0
Leelanau (MI)	8	0
Total	**72**	**26,109**

Table 3.3. Passport data of hop (*Humulus* spp.) accessions from USDA Germplasm Resources Information Network (GRIN) used in initial disease screening assays assessing inter- and intraspecific variation to foliar resistance to hop downy mildew using a detached leaf assay.

Plant Introduction #	**Origin**	**Taxon**
617473	United States	*H. japonicus*
635247	United States	*H. l.* var. *lupuloides*
635226	United States	*H. l.* var. *neomexicanus*
635261	Kazakhstan	*H. l.* var. *lupulus*
617282	Breeding	*H. l.* var. *lupulus* cv. Pacific Gem

Table 3.4. Passport data of hop (*Humulus lupulus* L.) accessions from USDA Germplasm Resources Information Network (GRIN) used for detached leaf assays.

GRIN Accession (PI)	GRIN Inventory ID	Num. Plants Evaluated	Taxon (var.)	Origin
635242	1001	1	lupuloides	North Dakota, USA
635243	1002	2	lupuloides	North Dakota, USA
635244	1003	5	lupuloides	North Dakota, USA
635246	1005	3	lupuloides	North Dakota, USA
635247	1006	3	lupuloides	North Dakota, USA
635252	1011	2	lupuloides	Saskatchewan, CA
617471	1019	1	pubescens	Missouri, USA
635261	1024	1	lupulus	Kazahkstan
635262	1025	1	lupulus	Kazahkstan
635285	1175	1	lupuloides	North Dakota, USA
635288	1178	3	lupuloides	North Dakota, USA
635302	1193	2	lupuloides	Saskatchewan, CA
635304	1195	2	lupuloides	Saskatchewan, CA
635312	1203	1	lupuloides	Saskatchewan, CA
635317	1208	4	lupuloides	Saskatchewan, CA
635322	1213	1	lupuloides	Manitoba, CA
635351	1250	4	lupuloides	Saskatchewan, CA
635359	1258	1	lupuloides	Saskatchewan, CA
635362	1261	1	lupuloides	Saskatchewan, CA
635365	1264	1	lupuloides	Saskatchewan, CA
635368	1267	3	lupuloides	Saskatchewan, CA
635371	1270	1	lupuloides	Saskatchewan, CA
635379	1280	3	lupuloides	Saskatchewan, CA
635390	1291	2	lupuloides	Saskatchewan, CA
635402	1305	1	lupuloides	Saskatchewan, CA
635418	1321	2	lupuloides	Saskatchewan, CA
635431	1335	4	neomexicanus	Colorado, USA
635433	1338	6	neomexicanus	Colorado, USA
635437	1342	1	neomexicanus	Colorado, USA
635458	1363	1	neomexicanus	Colorado, USA
635463	1368	1	neomexicanus	Colorado, USA
635477	1382	5	neomexicanus	New Mexico, USA
635482	1388	1	neomexicanus	New Mexico, USA
635226	1426	6	neomexicanus	Arizona, USA

Table 3.5. List of hop (*Humulus lupulus* L.) accessions common between Kralj *et al.* (1998) and Woods and Gent (2016). Average trait values for proportion of infected shoots and percent foliar disease as reported in their respective studies.

Cultivar	Prop. Infected Shoots	Foliar Disease (%)
Ahil	0.15	30
Apolon	0.04	20
Atlas	0.19	34
Aurora	0.18	13
Backa	0.22	52
Blisk	0.04	23
Bobek	0.24	24
Brewers Gold	0.15	30
Bullion	0.29	32
Cascade	0.26	12
Cekin	0.02	23
Celeia	0.05	17
Cerera	0.02	14
Comet	0.23	44
Eastwell Golding	0.2	37
First Choice	0.31	36
Fuggle N	0.09	24
Galena	0.27	34
Hallertauer Gold	0.003	22
Hallertauer Magnum	0.01	22
Hallertauer Mittelfrueh	0.39	32
Hallertauer Tradition	0.001	18
Hueller Bitter	0.02	17
Keyworths Midseason	0.33	39
Kirin II	0.23	45
Kitamidori	0.19	27
Nadwislanka	0.24	50
Northern Brewer	0.28	23
Nugget	0.35	42
Omega	0.03	27
Orion	0.001	22
Perle	0.001	30
Pride Of Ringwood	0.13	30
Saazer	0.31	36
Savinja Golding	0.13	14
Southern Brewer	0.22	37
Spalter Select	0.11	37
Tardif De Bourgogne	0.46	36
Willamette	0.15	35
Wye Challenger	0.04	18
Wye Saxon	0.28	24
Wye Target	0.31	49
Wye Viking	0.02	17
Wye Zenith	0.05	22

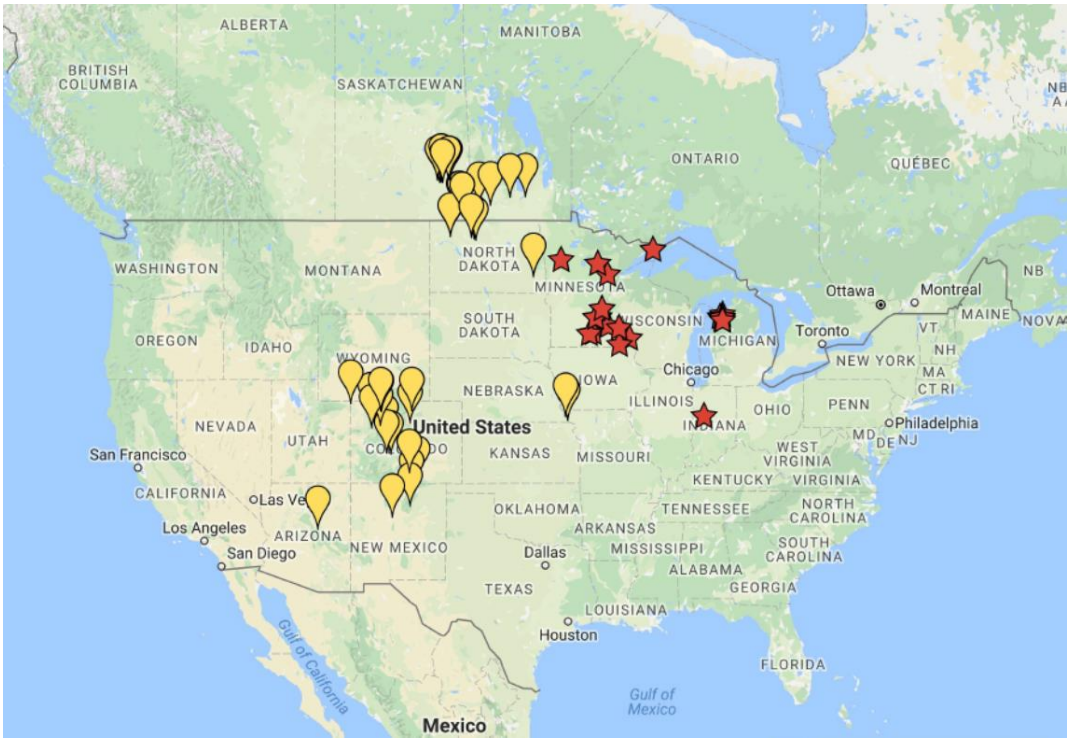

Figure 3.1. Distribution of hop (*Humulus* spp.) germplasm collections, including USDA Germplasm Resources Information Network (GRIN) (yellow teardrops) and privately-collected specimens (red stars), used for detached leaf assays (Table 3.2 and 3.3).

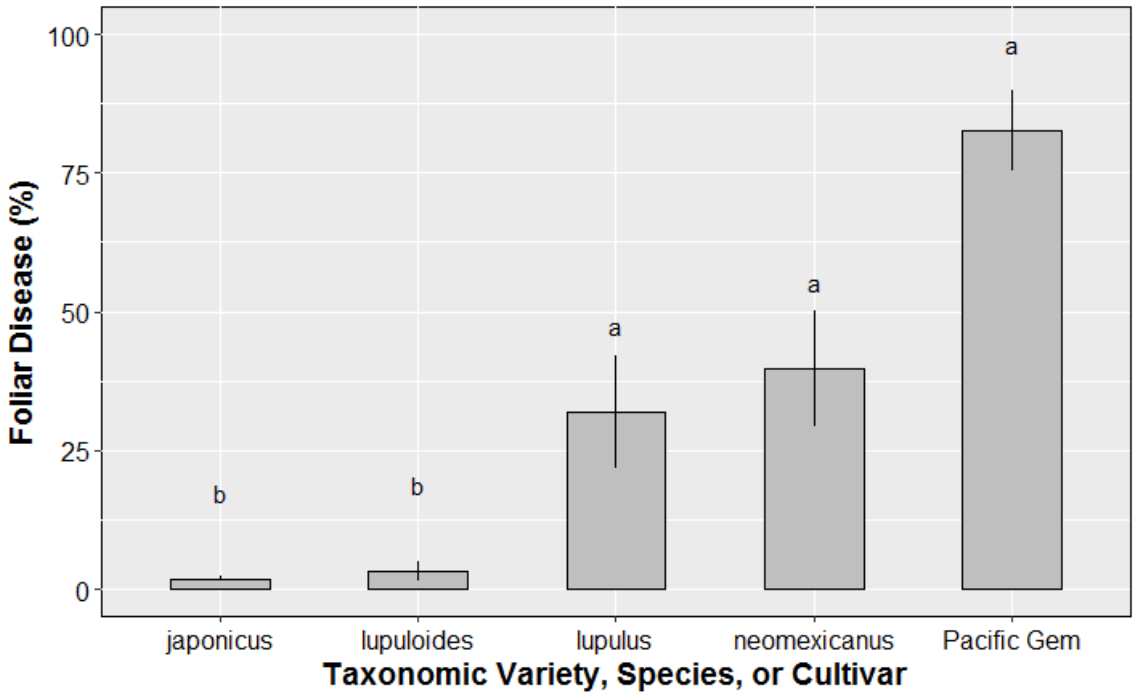

Figure 3.2. Percent foliar hop downy mildew disease development of inter- and intraspecific groups of wild hop (*Humulus* spp.) plants from USDA Germplasm Resources Information Network (GRIN). A single replicate of five leaves from each plant was collected in the early morning and each leaf was placed abaxial surface up onto a sterile, moist paper towel inside of a 90 mm Petri dish. The abaxial leaf surface was misted using a handheld atomizer with approximately 1 mL (50,000 sporangia) of prepared *P. humuli* inoculum. Plates were placed in a growth chamber at 20 °C with a 14 h photoperiod and incubated for 7 days following inoculation. The leaves were then digitally scanned using a Cannon LiDE 1100 flatbed scanner, images were uploaded into APS ASSESS v2.0 and the percent diseased leaf area was determined using standard settings. Means and standard error (SE) are displayed (n = 6). Letters above SE bars indicate significance groupings as determined by Tukey's HSD; bars with the same letter do not differ significantly from one another (F= 22.567, P= <0.0001).

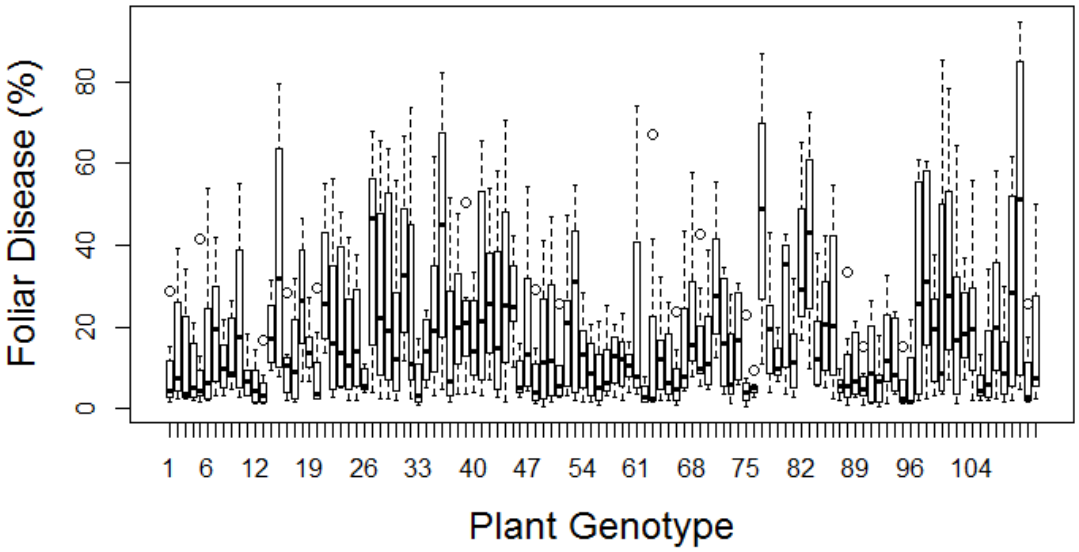

Figure 3.3. Percent foliar hop downy mildew disease development of 112 randomly selected wild hop (*Humulus lupulus* L.) genotypes from USDA Germplasm Resources Information Network (GRIN) and privately-collected specimens. A single replicate of five leaves from each plant was collected in the early morning and each leaf was placed abaxial surface up onto a sterile, moist paper towel inside of a 90 mm Petri dish. The abaxial leaf surface was misted using a handheld atomizer with approximately 1 mL (50,000 sporangia) of prepared *P. humuli* inoculum. Plates were placed in a growth chamber at 20 °C with a 14 h photoperiod and incubated for 7 days following inoculation. The leaves were then digitally scanned using a Cannon LiDE 1100 flatbed scanner, images were uploaded into APS ASSESS v2.0 and the percent diseased leaf area was determined using standard settings. A box and whisker plot is displayed (n = 8) that identifies medians, interquartile ranges, minimum values, maximum values, and outliers for each hop genotype assessed. Outliers are represented by empty black circles.

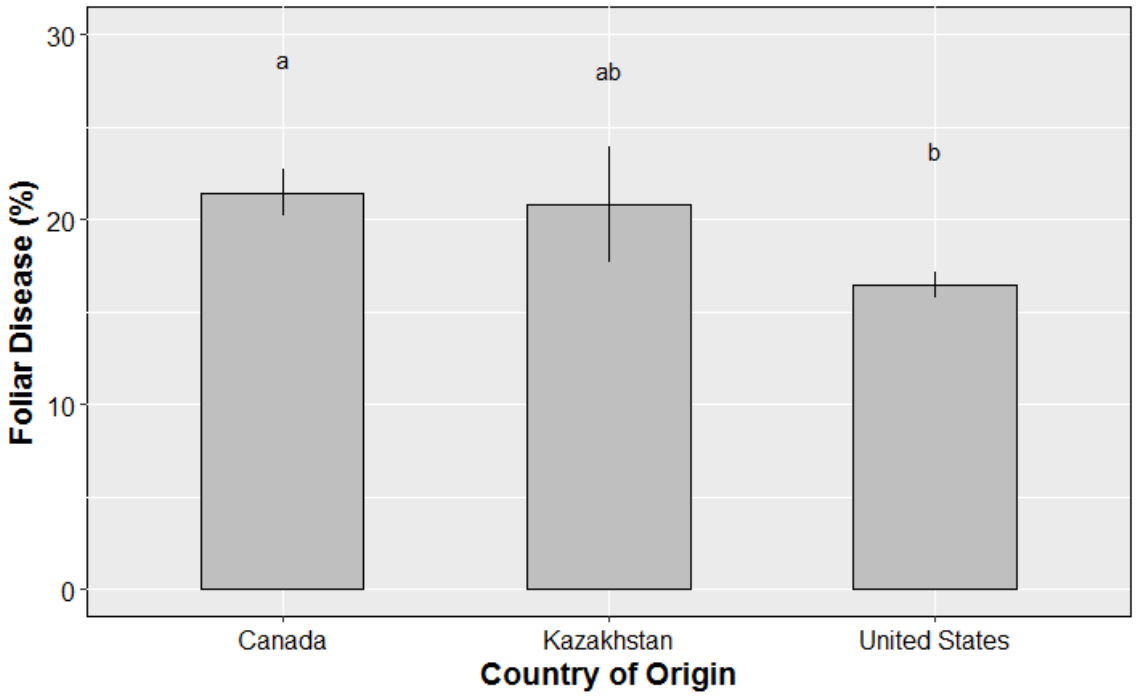

Figure 3.4. Percent foliar hop downy mildew disease development of wild hop (*Humulus lupulus* L.) plants based upon grouping by country of origin from USDA Germplasm Resources Information Network (GRIN) and privately-collected specimens. A single replicate of five leaves from each plant was collected in the early morning and each leaf was placed abaxial surface up onto a sterile, moist paper towel inside of a 90 mm Petri dish. The abaxial leaf surface was misted using a handheld atomizer with approximately 1 mL (50,000 sporangia) of prepared *P. humuli* inoculum. Plates were placed in a growth chamber at 20 °C with a 14 h photoperiod and incubated for 7 days following inoculation. The leaves were then digitally scanned using a Cannon LiDE 1100 flatbed scanner images were uploaded into APS ASSESS v2.0 and the percent diseased leaf area was determined using standard settings. Means and standard error (SE) are displayed (n = 8). Letters above SE bars indicate significance groupings as

determined by Tukey's HSD; bars with the same letter do not differ significantly from one another (F= 3.4989, *P*= 0.0337).

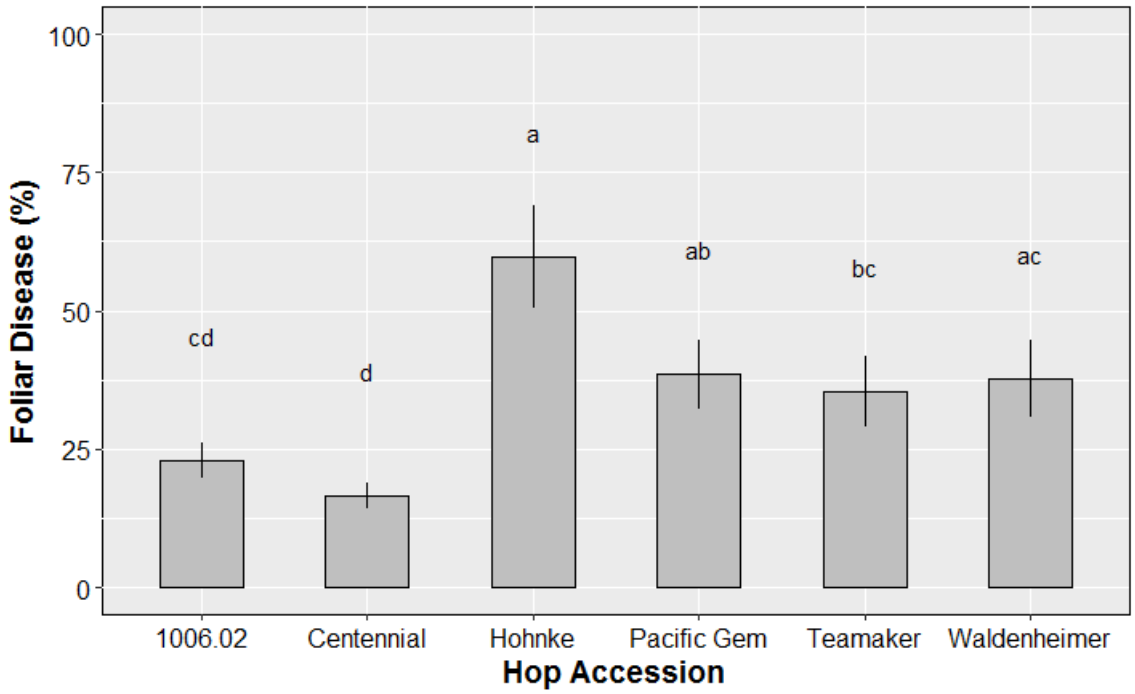

Figure 3.5. Percent foliar hop downy mildew disease development of six selected hop (*Humulus lupulus* L.) accessions consisting of three commercial cultivars (Centennial, Pacific Gem, and Teamaker) and three wild accessions (1006.02, Hohnke, and Waldenheimer). Three three-week old plants were inoculated as described above by lightly misting the abaxial surfaces of the leaves and incubated for 24 h at 20 °C after placing the whole plant in a plastic bag out of direct sunlight in a greenhouse. Following the incubation period, plants were removed from the plastic bags and placed into a greenhouse devoid of *P. humuli* with a 16 h photoperiod at 22 °C (± 3 °C) and fertilized once weekly with a solution of 400 ppm N. Two weeks following inoculation, five leaves were randomly-selected from each of three plants (replicates) and digitally-scanned using the previously described methods. This experiment was repeated six times. Means and standard error (SE) are displayed (n = 6). Letters above SE bars

indicate significance groupings as determined by Tukey's HSD; bars with the same letter do not differ significantly from one another (F= 13.659, *P*= <0.0001).

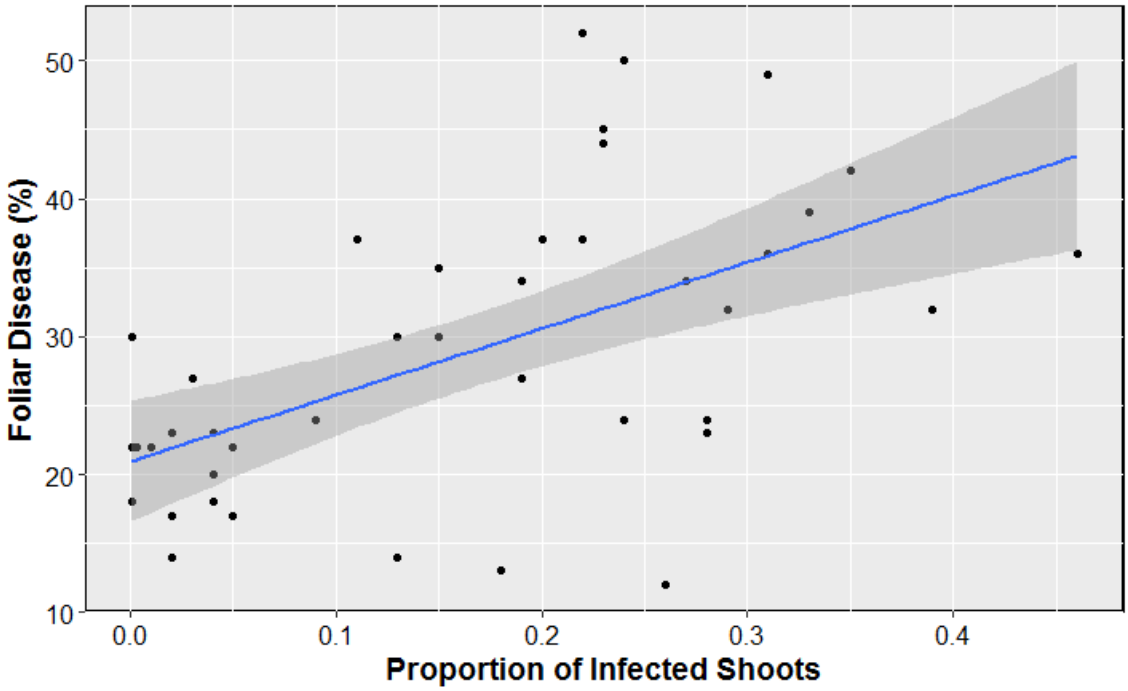

Figure 3.6. Correlation analysis of hop downy mildew phenotypes (*N* = 44) with data from Kralj *et al.*, 1998 and Woods and Gent, 2016. Kralj *et al.* (1998) evaluated leaves of ten plants of each hop accession in a non-sprayed hopyard and determined the average percent diseased leaf area during two evaluation periods. Woods and Gent (2016) determined the average proportion of infected shoots during three or four evaluation periods over three years. The blue line indicates the predicted mean and grey shaded area represents the 95% confidence interval.

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Appendix A

Evaluating nitrogen source and timing of applications on overall cone yield and brewing characters in commercial hopyards

Materials

30” soil probe

Poly-coated urea

Urea

Sustane®

Hop plants (cv. Cascade)

1000 µL pipette

500 µL microcentrifuge tube

Distilled water

LAQUA Twin Nitrate Meter (Spectrum Technologies, Aurora, IL)

Procedures

1. Three commercial hopyards in Minnesota (Elysian 44.25° N, -93.73° W; Ham Lake 45.27° N, -93.24° W; Shakopee 44.70° N, -93.53° W) were planted to the hop cv. Cascade. At each location, twenty 24” soil cores were collected and submitted to the UMN Soil Analysis Laboratory in St. Paul, MN.

2. On May 25th, 2015, two (Elysian) or three (Shakopee and Ham Lake) plants were treated with zero (control), 70 lbs N/acre, or 140 lbs N/acre, with nitrogen originating from one of three sources (urea, poly-coated urea, or Sustane®). Each treatment was incorporated into the top 6” of soil at each location. Additional phosphorous, potassium, or micronutrients were added as needed to standardize baseline nutrient levels.

3. Growers trained vigorously growing stems following each treatment and maintained each yard as per normal production practices.

4. Following treatments, hopyards were visited at two to three week intervals and five leaves and petioles per plant were collected at approximately two meters in height. Collected tissues were placed in a plastic Ziploc® bag and stored on ice in a cooler until processed within 24 – 48 hours.

5. Tissue extracts from each experimental plot were collected by pressing tissue samples with a garlic press over a small sheet of Parafilm®. Samples were then pipetted into 500 µL microcentrifuge tubes and frozen until further analysis.

6. On June 25th, 2015, an additional 70 lbs N/acre was added to the three previous 70 lbs N/acre treatments with the corresponding nitrogen source.

7. Three 0.1 mL samples of each tissue extract were placed on the sensor of a LAQUA Twin Nitrate Meter after thawing and followed by vortexing for 20 seconds. Samples were diluted with distilled water as needed for measurement.

8. At harvest, plants were cut at one meter above ground and inflorescences were harvested using a Bine Harvester 3060® (Gorst Valley Hops, Mazomanie, WI) or a mobile-type harvester. Overall yield was averaged across two (or three) plants in each

experimental treatment plot at each location. Moisture content was measured and standardized to 8%.

9. A 30 gram sample of dried hop inflorescences from each experimental plot was submitted to Western Michigan University for analysis of alpha and beta acids (co- and ad- respecitively) and total acid content.

Experimental Results

A mixed model analysis was carried out on the results of the data collected during 2015. Location and treatments were considered fixed effects while replicates nested within locations were considered a random effect. The response variables were yield, standardized to 8% moisture, and tissue nitrate concentration. Statistical analysis of overall plot yield indicated that locations were significantly different from each other ($F = 5.748$, $P = 0.0284$). Pairwise comparisons indicated that Ham Lake had significantly higher yields over when compared to Elysian ($P = 0.0272$) but only marginally higher yields compared to Shakopee ($P = 0.0999$). Statistical analysis of tissue nitrate concentration (averaged across all sampling periods) indicated that locations were significantly different from each other ($F = 31.470$, $P = 0.0008$). Pairwise comparisons indicated that Elysian had significantly lower average tissue nitrate concentrations than both Ham Lake and Shakopee ($P = 0.0007$; $P = 0.0056$ respectively) and that Ham Lake had only marginally higher average tissue nitrate concentration ($P = 0.0919$).

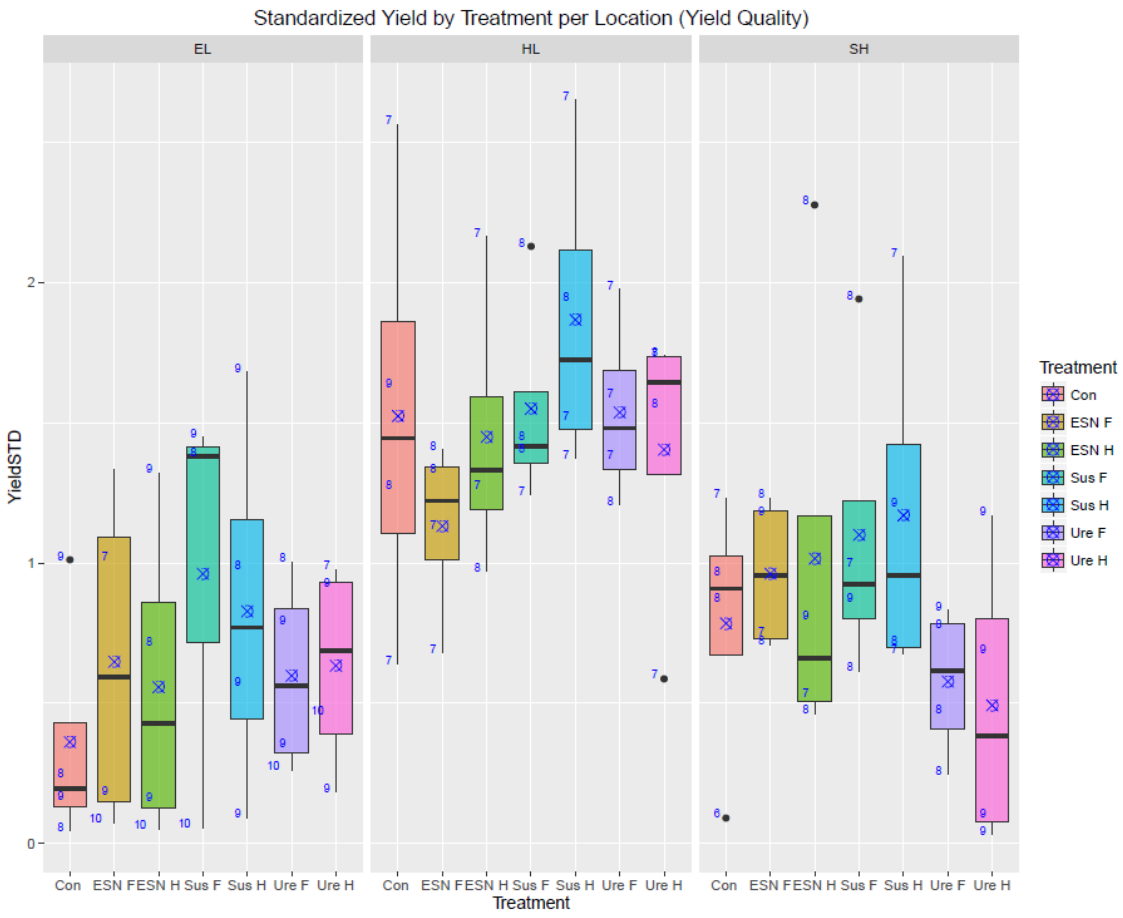

Figure A.1. Box and whisker plot of standardized yield (8% moisture) separated by treatments and locations. Treatments included zero nitrogen (control), two 70 lbs N/acre, or one 140 lbs N/acre with nitrogen originating from urea, poly-coated urea, or Sustane®. The split application of nitrogen was made five weeks apart.

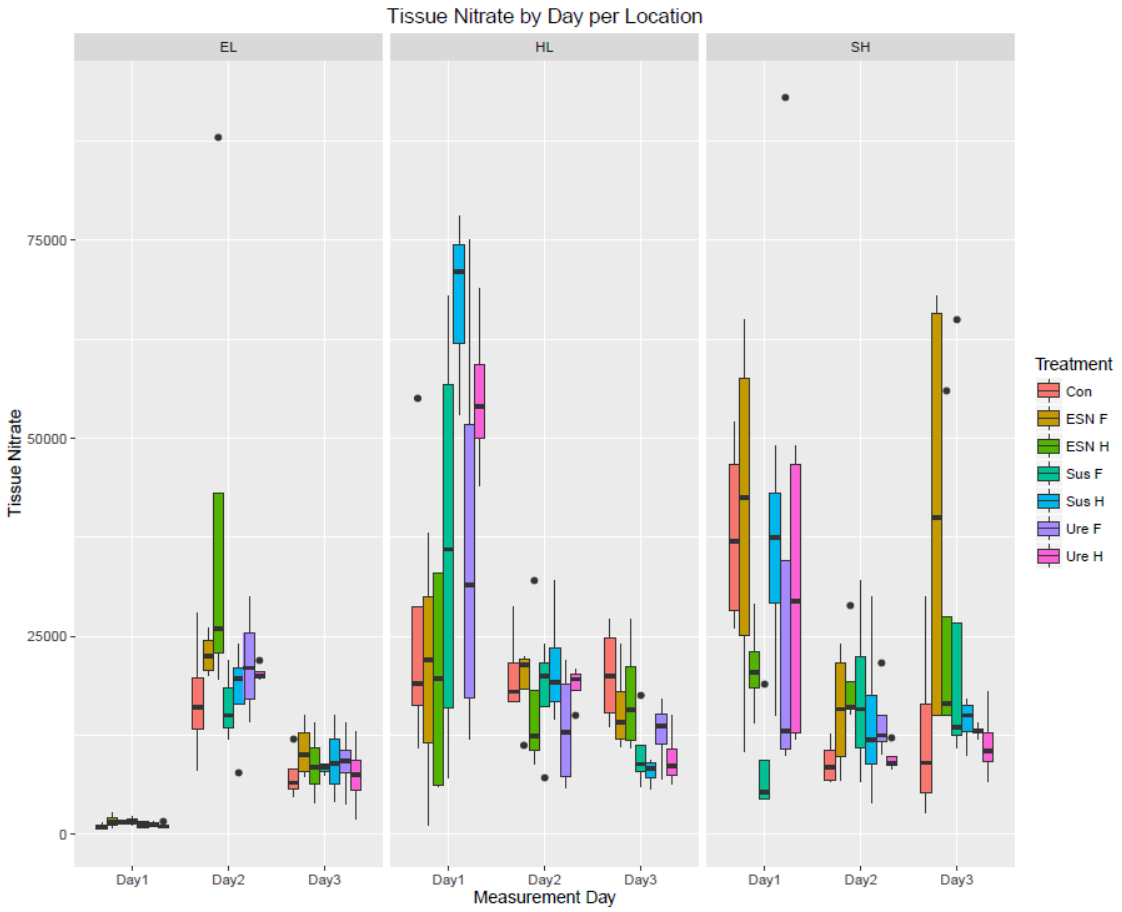

Figure A.2. Box and whisker plot of tissue nitrate separated by treatments, days, and locations. Treatments included zero nitrogen (control), two 70 lbs N/acre, or one 140 lbs N/acre with nitrogen originating from urea, poly-coated urea, or Sustane®. The split application of nitrogen was made five weeks apart.

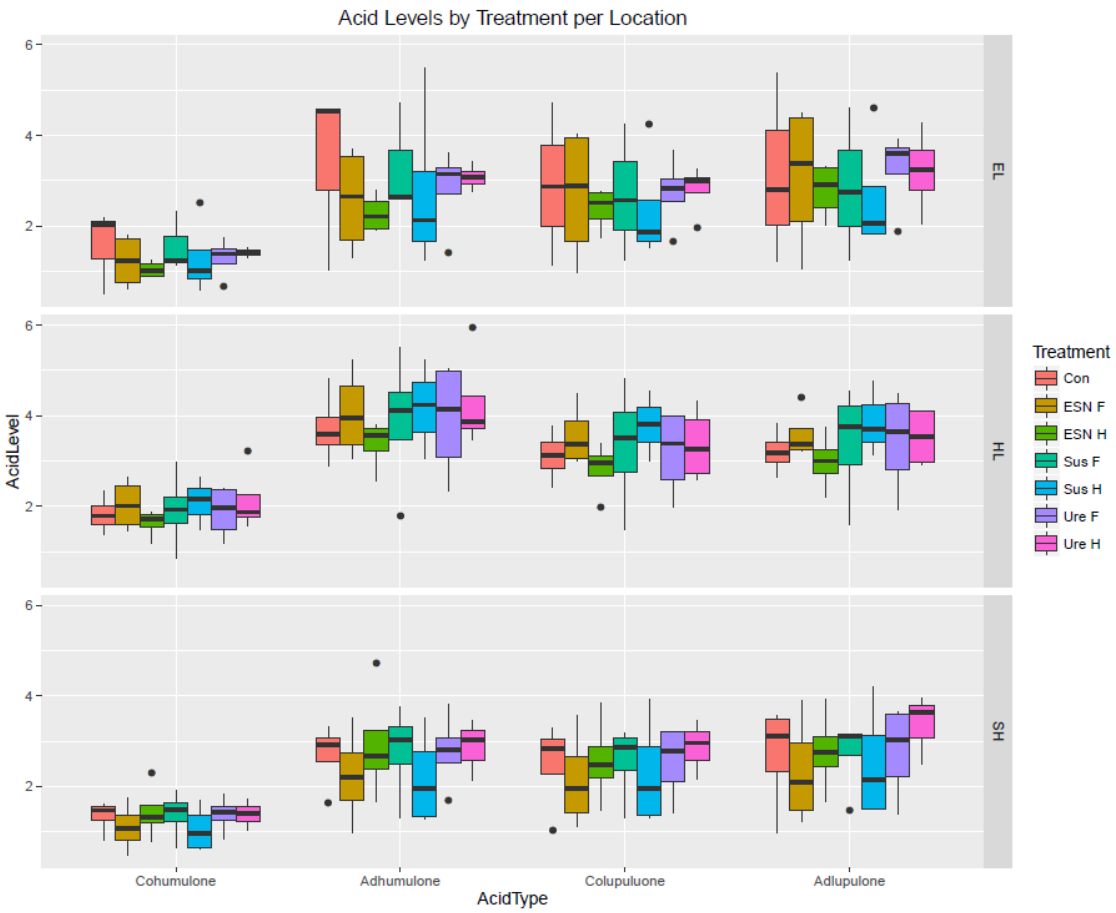

Figure A.3. Box and whisker plot of alpha and beta acids (co- and ad-, respectively) separated by treatments and locations. Treatments included zero nitrogen (control), two 70 lbs N/acre, or one 140 lbs N/acre with nitrogen originating from urea, poly-coated urea, or Sustane®. The split application of nitrogen was made five weeks apart.

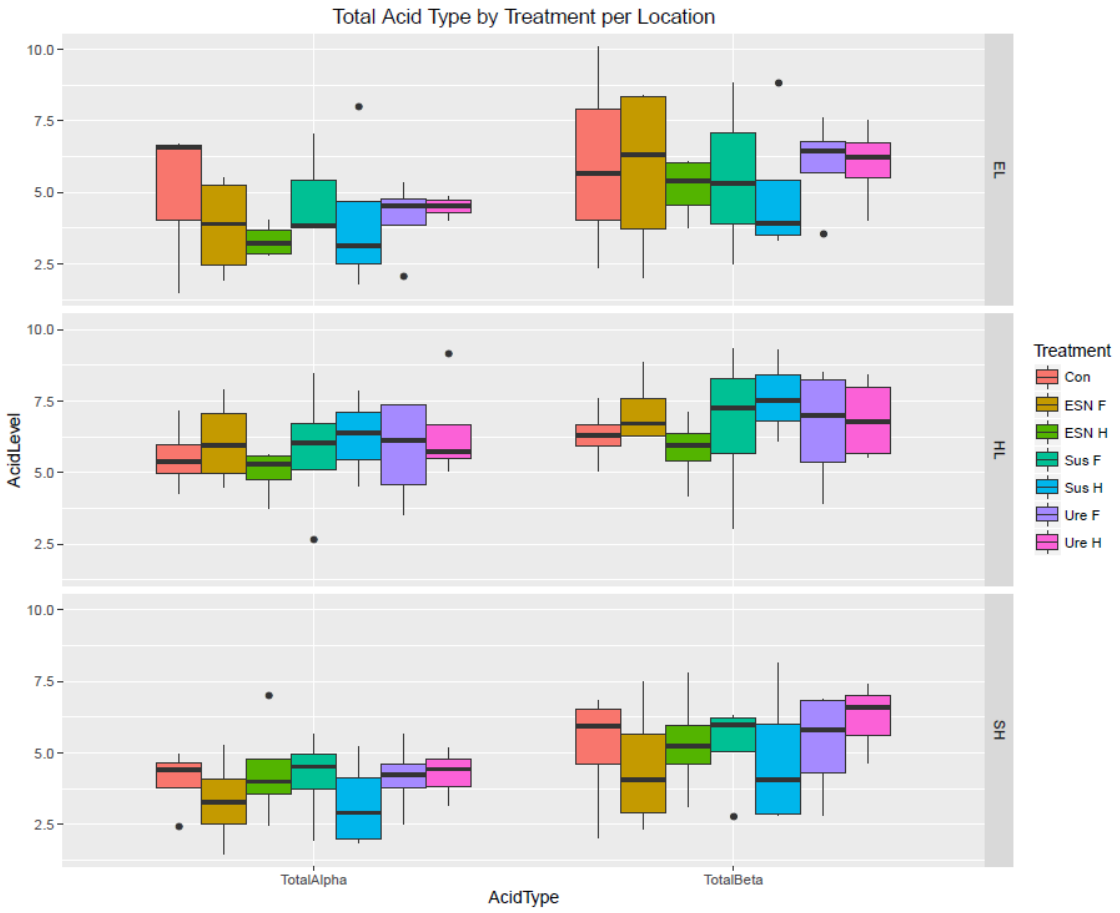

Figure A.4. Box and whisker plot of total alpha and beta acids separated by treatments and locations. Treatments included zero nitrogen (control), two 70 lbs N/acre, or one 140 lbs N/acre with nitrogen originating from urea, poly-coated urea, or Sustane®. The split application of nitrogen was made five weeks apart.

Appendix B

Infestation of hop seed (*Humulus lupulus*) by chasmothecia of the powdery mildew fungus, *Podosphaera macularis*

Reprinted from Claasen *et al*., 2017, Infestation of hop seed (*Humulus lupulus*) by chasmothecia of the powdery mildew fungus, *Podosphaera macularis*, Plant Disease, https://doi.org/10.1094/PDIS-03-17-0328-PDN

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Powdery mildew, caused by *Podosphaera macularis*, is responsible for large economic losses in hop (*Humulus lupulus*) in the primary production regions of the crop in the Pacific Northwestern U.S. (Gent *et al*., 2008). *Podosphaera macularis* is heterothallic, but to date only the MAT1-1 mating type has been confirmed in the Pacific Northwest (Wolfenbarger *et al.*, 2015) and ascocarps of the fungus have not been observed in this region (Gent *et al*., 2006). In the autumn of 2015, seed was collected from wild hop plants at 7 locations in Minnesota for grow out and evaluation of various traits. Prior to planting, seeds were examined under low magnification (30-50×) and in 9 of the 11 seedlots, representing 4 of 7 locations, the seed was found to be externally infested with spherical to flattened, black chasmothecia. In infested lots, the number of seed bearing chasmothecia averaged 45% (range 5 to 89%; n = 107 to 200 seeds per lot). Scanning electron microscopy indicated chasmothecia had a mean diameter of 82µm and were shriveled with a concaved base. Chasmothecia were easily dislodged from the seed coat despite the appendages being embedded in a mat of pannose mycelium. Conidiophores and conidia were not observed. The morphological characters were consistent with the genus *Podosphaera* (Braun *et al.*, 2002; Wolfenbarger *et al.*, 2015). Chasmothecia were

confirmed as *P. macularis* by extracting DNA from 10 to 15 seeds from each of 6 seedlots using a DNeasy PowerSoil Kit (Qiagen, Carlsbad, CA) and amplifying and sequencing the MAT1-1 and MAT1-2 idiomorphs as described by Wolfenbarger et al. (2015). The sequences obtained for MAT1-1 and MAT1-2 were identical among the extractions of the 6 seedlots. Standard nucleotide BLAST searches in GenBank indicated that the sequences were 97% similar to MAT1-1 (accession KJ922755.1) and 100% similar to MAT1-2 (accession KJ741396.1) sequences of *P. macularis*. To our knowledge, this is the first report of infestation of hop seed by chasmothecia of *P. macularis*. Current quarantine laws that restrict import of planting materials for hop into Idaho, Oregon, and Washington explicitly exempt seed. However, seed infested with chasmothecia may spread the pathogen, potentially introducing novel isolates and mating types of the pathogen. Seed transmission of powdery mildew organisms is scarcely reported (Jarvis *et al*., 2002), and studies are needed to determine the risk of disseminating *P. macularis* on infested seed. Until such information is available, caution is advised when moving seed from regions where powdery mildew occurs.

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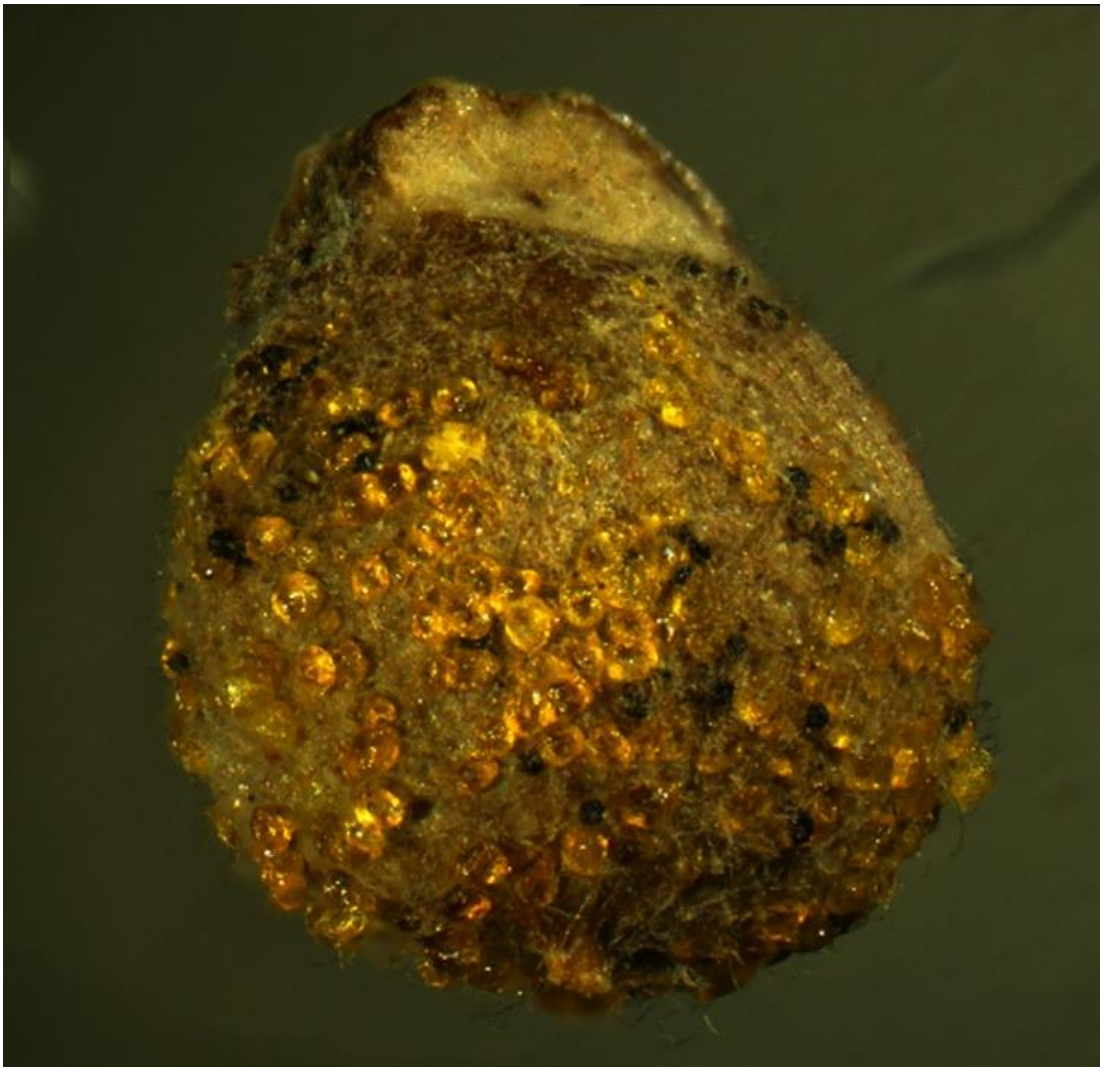

Figure B.1. Seed from a wild hop plant (*Humulus lupulus* L.) with numerous, black, spherical chasmothecia of *Podosphaera macularis* and extensive mycelial colonization on the seed coat. The larger yellow structures are lupulin glands.

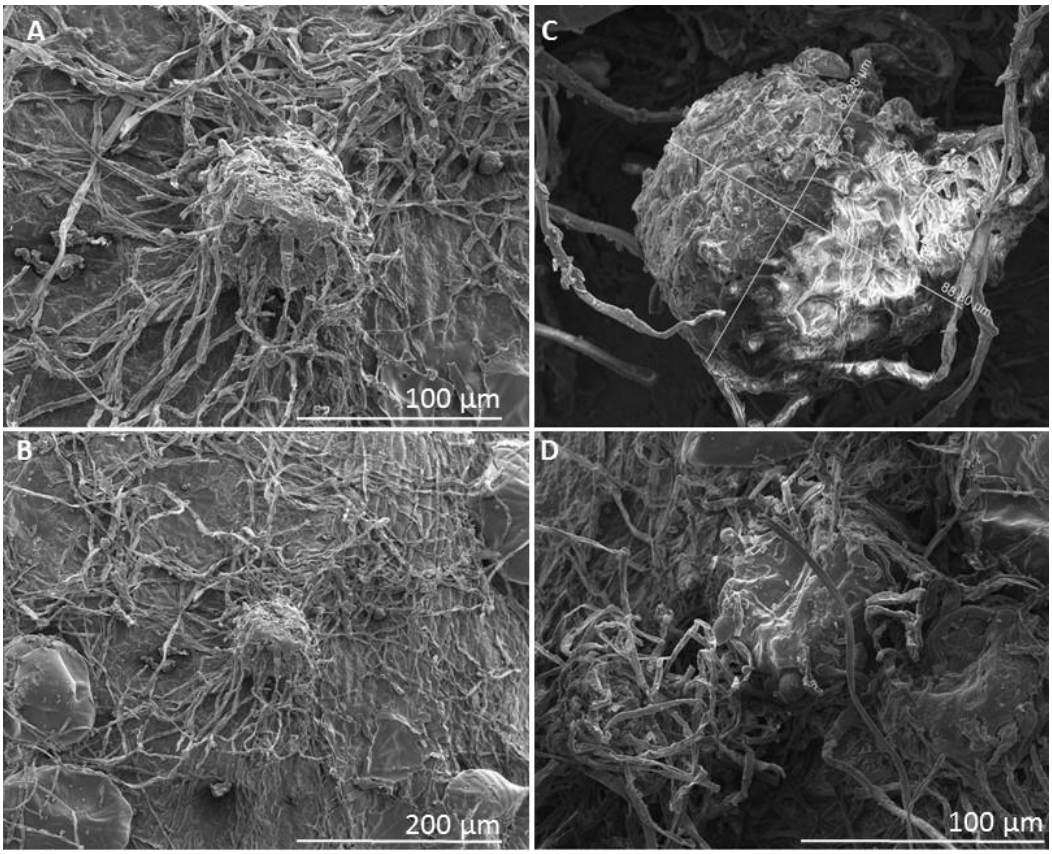

Figure B.2. Scanning electron micrographs of chasmothecia of *Podosphaera macularis* on hop seed. **A.** Chasmothecium with myceloid appendages on seed coat. **B.** Chasmothecium on seed coat with appendages embedded in pannose mycelium. Larger, non-descript structures are lupulin glands. **C.** Close-up with measurements of the diameter of an ascocarp. **D.** Shriveled ascocarps with concaved basal portions.