AN ABSTRACT OF THE THESIS OF

Sierra N. Wolfenbarger for the degree of Master of Science in Botany and Plant Pathology presented on May 9, 2014.

Title: Identification and Distribution of the Mating-Type Locus and Development of Cleistothecia of *Podosphaera macularis*.

Abstract approved:

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David H. Gent

Podosphaera macularis, causal agent of hop powdery mildew, is known to produce

cleistothecia (syn. chasmothecia) in eastern North America and Europe, but ascocarps

are not reported from the Pacific Northwestern region of North America. Reasons for

the apparent absence of cleistothecia in the Pacific Northwest are unknown. We

established that *P. macularis* is heterothallic and that ascocarp ontogeny, maturation,

dehiscence, and ascopore infection proceeds similarly to other powdery mildew fungi.

Genome sequencing of a *MAT1-1* isolate revealed the structure of the *MAT1* locus and

presence of *MAT1-1-3* demonstrating further similarities to other powdery mildew

fungi. PCR assays with primers designed from conserved domains of the *MAT1*

idiomorphs were developed to characterise the frequency of idiomorphs in populations

of *P. macularis.* Amongst 317 samples of *P. macularis* collected during 2012 and

2013 from the Pacific Northwest only the *MAT1-1* idiomorph was found. In contrast,

among 56 isolates from the eastern United States and Europe, *MAT1-1* and *MAT1-2*

idiomorphs were detected at equivalent frequencies. At temperatures representative of

late season conditions in the Pacific Northwest, cleistothecia formed readily when a *MAT1-1* isolate from the Pacific Northwest was paired with a *MAT1-2* isolate collected from outside the region. Although these findings do not encompass all climatic, geographic, or temporal barriers that could inhibit the formation of cleistothecia, the current absence of the ascigerious stage of *P. macularis* in the Pacific Northwest could be explained by the absence of the *MAT1-2* mating type idiomorph.

Identification and Distribution of the Mating-Type Locus and Development of
Cleistothecia of *Podosphaera macularis*

by

Sierra N. Wolfenbarger

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Sierra N. Wolfenbarger, Author

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CONTRIBUTION OF AUTHORS

David Gent was involved with all aspects of these studies. David Gadoury provided assistance with documentation of cleistothecia development and performed viability and infection assays with ascospores in Geneva, NY. Brain Knaus and Nik Grünwald provided guidance and assistance in the assembly and analysis of the Illumina data. Megan Twomey assisted in trouble shooting molecular assays and the identification of the mating type idiomorphs.

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX FIGURES

Chapter 1: Introduction and Literature Review

Rationale for Research

Humulus lupulus, the common hop, has been grown in the Pacific Northwestern United State since the early 1900s. An economically important pathogen of hop is *Podosphaera macularis*, which causes powdery mildew. In the Pacific Northwestern U.S. the ascigerious overwintering stage of *P. macularis* is not observed, forcing the pathogen to overwinter by bud perennation or to find refuge in greenhouses producing hop. Understanding why cleistothecia have not been observed or do not form is important because the approach the pathogen uses to overwinter has implications for management practices and tactics, such as pruning in the late spring. It is thought that the formation of the ascigerious stage in *P. macularis* requires two mating types (heterothallism) and that the lack of the ascigerious stage in the Pacific Northwest is due to the absence of one of the mating types in the population. However, this hypothesis is speculative and the reason for the lack of the sexual stage in this population has not been determined. There is also little evidence to confirm the heterothallic nature of *P. macularis*. Understanding the biology of the initiation of the sexual stage would clarify the apparent absence of the ascigerious stage of the fungus in the Pacific Northwest and could potentially inform quarantine regulations.

Hop, *Humulus lupulus* L.

Humulus lupulus L., the common hop, is a dioecious plant grown between latitudes of approximately 35° and 55° south and north due to day length requirements of the plant for flowering. *H. lupulus,* along with two other species of *Humulus*, *H. japonicus* and *H. yannanensis*, is in the family Cannabaceae. All three species are thought to have originated in China, where all three are found naturally (Neve, 1991). Little is known about *H. yannanensis* except that it is perennial and thought to occur at high altitudes in southern China (Neve, 1991). *H. japonicus* is an annual species, although some evidence suggests that in certain environments *H. japonicus* may persist for multiple seasons. This species has no brewing value because the cones produce very few lupulin glands but it is occasionally grown as an ornamental to provide a leafy screen (Neve, 1991).

The earliest records of *H. lupulus* being cultivated are from the 8th century, when French and German monks grew hops, presumably for their medicinal value (Burgess, 1964). Today, hop cones are primarily used to preserve and add flavor, bitterness, and aroma to beer. In the United States, hop production began on the east coast but due to economic factors and disease pressure commercial production gradually shifted to the western U.S. (Barth *et al.,* 1994). At present, hops are produced mainly in the Pacific Northwest (Washington, Oregon and Idaho) where in 2013 approximately 69.3 million pounds of hops worth $249,000,000 were harvested (USDA, 2013).

Hop powdery mildew caused by *Podosphaera macularis* Braun & Takamatsu (formerly *Sphaerotheca macularis* (Wallr. :Fr.) Lind, syn. *S. humuli* (DC.) Burrill) is one of the oldest and economically important diseases of hop, and is known in Germany as 'Echter Mehltau' or true mildew (Neve, 1991). Despite the hop industry being present in the Pacific Northwestern United States since the 1900s (Barth *et al.,* 1994), powdery mildew was not reported in fields in the Pacific Northwest until 1997 (Ocamb *et al.,* 1999). Hop powdery mildew was first observed in the Pacific Northwest in 1996 in a greenhouse in the lower Yakima Valley in Washington (Mahaffee *et al.,* 2003). After this initial discovery, in 1997 powdery mildew was discovered in other greenhouses and in hop fields in Washington (Ocamb *et al.,* 1999; Turechek *et al.,* 2001; Mahaffee *et al.,* 2003). In 1998 the pathogen had spread further to Oregon and Idaho, thereby encompassing the entire hop industry in the Pacific Northwest (Ocamb *et al.,* 1999; Mahaffee *et al.,* 2003). The disease now occurs annually in all three states. If left unmanaged powdery mildew is capable of destroying an entire crop and was partially responsible for driving the hop industry out of New York in the early 1900's (Blodgett, 1913; Barth *et al.,* 1994).

Powdery Mildew Fungi

Powdery mildews (Erysiphales, Ascomycota) are obligate parasitic fungi, meaning that the pathogens can only grow and reproduce on living host tissue. There are 16 genera of powdery mildews and at least 900 species recognized presently (Takamatsu, 2012). The disease name powdery mildew comes from the appearance of the asexual

stage of reproduction, producing conidia, which as appear as a conspicuous, light dusting of powder on the host. Despite species of powdery mildew fungi being specialized for a limited number of host plants, these fungi cause extensive economic loss on a large range of agricultural crops as well as ornamentals (Jarvis *et al.,* 2002).

In favorable conditions with no management powdery mildew pathogens may produce 20 generation in a growing season, with an infection from a single conidium resulting in as many as 3.53×10^5 conidia/cm^2 of colony area being produced and spread by wind (Blodgett, 1913). Due to this ability to produce large amounts of asexual inoculum and tolerance of the pathogens to a relatively wide range of environmental conditions, powdery mildews in an agricultural setting can limit the yield and quality of fruits, grains, and vegetable (Jarvis *et al.,* 2002). In field crops such as wheat and other cereals, powdery mildew diseases primarily affect yield by diminishing photosynthetic capacity (Gaunt, 1995). In these crops a measure of disease severity or healthy leaf area can be used to estimate crop damage, and empirical damage functions often indicate a negative linear relationship between powdery mildew severity and yield (Lipps & Madden, 1989). It can be more difficult to estimate the amount of crop damage and economic loss caused by powdery mildew in commodities that are used directly by buyers, such as in fruits and vegetables, where the harvested products are judged on appearance (Jarvis *et al.,* 2002).

Management of Powdery Mildew

Management of powdery mildew diseases is unique to each pathosystem and can include, but is not limited to, genetic resistance in the host, manipulation of spacing of plants and density of foliage in a field, removal of over-seasoning inoculum through management practices such as pruning or tilling, and fungicide applications, with the impact of powdery mildew on crop loss and yield influencing the approach of these management strategies (Jarvis *et al.,* 2002). As an example, *Blumeria graminis* causes powdery mildew on wheat, barley and other grasses and was voted to be one of the top ten fungal pathogens by Molecular Plant Pathology in 2012 (Dean *et al.,* 2012). Control of these diseases is achieved by a combination of fungicide sprays and disease resistant cultivars. Management approaches are continually updated as strains of the pathogen develop resistance to fungicides and overcome host resistance (Dean *et al.,* 2012). Another well-studied pathogen, *Erysiphe necator*, causal agent of grape powdery mildew, is one of the most widespread and destructive diseases of grape. Damage from *E. necator* is caused both by decreased yield owing to impairing photosynthetic ability of the plant and by decreasing quality of the fruit if berries become infected (Jarvis *et al.,* 2002; Calonnec *et al.,* 2004). Powdery mildew on grape is controlled by canopy manipulation and multiple fungicides applications in a year, resulting in substantial expenses to the growers of susceptible grape cultivars. However, due to the popularity of select susceptible cultivars of grape (i.e., 'Chardonnay', 'Riesling', and 'Pinot Noir') resistant cultivars do little to limit severity of powdery mildew and the expense of controlling the disease (Gadoury *et al.*, 2003).

In hop, damage from *P. macularis* is thought to be solely from the decreased quality and quantity of the cones (Royle, 1978; Neve, 1991; Gent *et al.,* 2008; Gent *et al.,* 2013). *P. macularis* can cause discoloration, malformation and even abortion of developing cones. Coley-Smith (1964) observed that the degree of cone discoloration was related to the severity of powdery mildew on the cone. If cleistothecia form, the cone can turn a black color, making the appearance undesirable to brewers (Royle, 1978). The presence of powdery mildew on cones has also been known to cause early ripening of cones (Coley-Smith, 1964; Mahaffee *et al*., 2009) due to an apparent acceleration of maturity that may lead to indirect yield damage due to shatter during harvest (Gent *et al.,* 2013).

Cone maturation and shattering from powdery mildew is associated with an increase in dry matter (Gent *et al.,* 2013). Dry matter content is a measure of maturity of cones and a typical desirable range is typically between 22 and 24% for most cultivars (Rybáček, 1991). The optimal dry matter is cultivar-dependent and influenced by field-specific factors such as soil type and irrigation practices, however, the presence of powdery mildew additively increases the rate of cone desiccation and dry matter accumulation from other factors.

Alpha acids are the most important of the bittering acids produced by hop, as these soft resins are essential for brewing (Neve, 1991; Hysert, 2009). Some cultivars of hop are grown purely for the extraction of alpha acids and because of this yield of alpha acids is generally more important than cosmetic appearance of the cones in such cultivars (Gent *et al.,* 2013). Retention of alpha acids and cone appearance, especially

color, are influenced by cone maturity, as measured by dry matter content of the cone at harvest. The overall dry matter content of cones at harvest is linked to yield of alpha acids, and therefore powdery mildew may cause indirect losses mediated by accelerated dry matter accumulation, depending on harvest timing (Gent *et al.,* 2013).

An important management strategy of powdery mildew on hop is the removal of buds and plant growth in the upper 5 to 10 cm of soil in early spring. This practice is referred to as crowning or pruning and effectively delays the initial spread of the disease due to removal of initial inoculum and changes in subsequent development of foliage (Turechek *et al.,* 2001; Mahaffee *et al.,* 2003; Gent *et al.,* 2008; Gent *et al.,* 2012). The quality of spring pruning is associated with overall severity of powdery mildew outbreaks on leaves and the incidence of cones with powdery mildew at harvest in the Pacific Northwest (Gent *et al.,* 2012). Fungicide applications are also applied to reduce damage caused by *P. macularis* (Turechek *et al.,* 2001; Mahaffee *et al.,* 2003). It was observed early on that fungicide sprays later in the season did not protect the crop as much as early applications (Blodgett, 1913).

Leaves of hop develop ontogenic or age-related resistance to powdery mildew (Royle, 1978), and until recently it was thought that the cones did not develop ontogenic resistance (Mahaffee *et al.,* 2003; Gent *et al.,* 2008). However, studies by Seigner *et al.* (2003) hinted that more mature cones might be less susceptible to powdery mildew. The studies by Seigner *et al.* (2003) were conducted using detached tissue and it was not possible to test all developmental stages. Other studies utilizing cones produced under more natural conditions in the field have since been conducted

and indicate hop cones develop partial ontogenic resistance (Woods *et al.,* 2010; Wolfenbarger *et al.,* 2013). Cones become progressively less susceptible to powdery mildew with maturity, but do not appear to become completely resistant to the disease. Because of this, the necessity of protection of cones through bloom and early stages of cone development (stage II of cone development as described by Kavalier *et al.,* 2011) with fungicides is critical to maintain cone quality and yield. However, fungicide treatments may not be necessary after the periods of greatest juvenile susceptibility provided cones are harvested before extreme over-maturity (Gent *et al.,* 2013).

Powdery mildew resistant cultivars are also utilized in certain locations. Salmon (1919) was the first to record resistance to powdery mildew in hop, indicating that two types of resistance were present; an immunity that resulted in no infection in the greenhouse (now known as qualitative resistance) and a 'semi-immunity' resulting in blisters on leaves that bore mycelia of the fungus present but did not sporulate (now known as quantitative resistance) (Royle, 1978). Since then, resistance to powdery mildew has been a main objective in breeding programs around the world and deployment of resistant cultivars has been used to assist in disease management. The breeding program at Wye College described multiple major gene resistances in hop by examining the range of responses of several hop cultivars to infection when challenged with several isolates of *P. macularis* (Royle, 1978). Royle (1978) stated that seven major resistant genes are described or proposed in hop, designated R_B, R_1, R_2, R_3, R_4, R_5, and R_6, with corresponding virulence in the pathogen designated V_B, V_1, V_2, V_3, V_4, V_5, and V_6, which has been confirmed by Darby (2013).

Epidemiology and Infection Process

The ability of powdery mildew fungi to produce asexual spores, conidia, drives

epidemics of powdery mildews during a growing season. Conidia are wind-dispersed

and do not require a moist period to infect as other fungi commonly do (Bushnell,

2002). Conidia production is influenced by a number of environmental factors

including light, temperature, humidity and rainfall (Yarwood, 1957; Neve, 1991;

Jarvis *et al.,* 2002; Mahaffee *et al.,* 2003; Takamatsu, 2004; Aslaf *et al.,* 2012). Each

factor may have both a direct effect on the pathogen and an indirect effect by its

influence on the host. Effects on the host plant in turn may impact the pathogen.

Infection, whether from a conidium or ascospore, by powdery mildew fungi

involves a complex process initiated almost immediately after contact with a

susceptible host plant (Green *et al.,* 2002). The beginning of germination is the

initiation of a germ tube that forms into specialized hyphae known as an appressorium,

which then forms a penetration peg. If these specialized hyphae are successful in

penetrating the host barrier to the epidermal cell then another specialized feeding

structure called a haustorium forms inside the host cell. Haustoria invaginate the

cytoplasm, allowing the fungus to obtain nutrients from the host (Green *et al.,* 2002;

Glawe, 2008).

After infection, hyphae elongate and branch, forming circular colonies. Most

hyphae grow superficially on their host with the exception of the tribe Phyllactinieae

that can grow inside host tissue (Green *et al.,* 2002). Hyphae eventually give rise to

conidia produced in chains known as conidiophores, which usually begin to form

several days after infection (Glawe, 2008). This can make management of powdery mildew fungi difficult, because by the time mildew symptoms are observed in the field, the fungi has had several days or longer to initiate and spread, making the epidemic difficult to control (Caffi *et al.,* 2013).

In hop there is a period early in the season when secondary infection rates of *P. macularis* are low due to unfavorable environmental conditions (Royle, 1978). Temperature is known to influence the success of infection, sporulation, and disease development in *P. macualaris* (Mahaffe *et al.,* 2003; Peetz *et al.,* 2009). Infection is possible at temperature ranging from 12 to 27ºC (Mahaffe *et al.,* 2003; Peetz *et al.,* 2009). The fungus is sensitive to supra-optimal temperature, and two hours of exposure to temperatures of 30ºC can reduce infection severity by 50% or more. Temperature greater than 38ºC can cause colony death (Mahaffee *et al.,* 2003, Peetz *et al.,* 2009).

Perennation of Powdery Mildew Fungi

The severity of the previous season powdery mildew epidemic generally is associated with the severity of the current season epidemic because increased inoculum tends to increase the quantity of pathogen over-seasoning (Caffi *et al.,* 2012; Legler *et al.,* 2013). The obligate biotrophic nature of powdery mildews restrict how powdery mildew fungi survive periods when their host is dormant, a process known as perennation. One means of perennation is to survive the off-season of the host in association living tissue of the host itself. This can be achieved by infecting quiescent

buds that emerged earlier in the season, allowing the pathogen to emerge the next

growing season with resumption of host growth (Royle, 1978; Jarvis *et al.,* 2002;

Glawe, 2008; Gadoury *et al.,* 2012). These infected buds result in the so-called 'flag

shoots'. In other cases, powdery mildew fungi will form an overwintering structure

known as a cleistothecium (syn. chasmothecium) during the growing season, allowing

the fungus to persist apart from living host tissue (Royle, 1978; Gadoury & Pearson,

1988; Jarvis *et al.,* 2002). A third option is that the host does not completely die,

allowing the powdery mildew mycelium to persist on the host in unfavorable

conditions (Glawe, 2008). An example of this is found in poinsettia where high

temperatures suppress but do not kill the powdery mildew fungus on this host.

Therefore symptoms of powdery mildew are not readily observed until the

environment becomes more favorable to the fungus (Celio & Hausbeck, 1997). This

mode of perennation is common in ornamentals that maintain foliage throughout the

year, such as *Nandina domestica* (Glawe, 2003). Some powdery mildew fungi may

have alternate hosts that can be infected when one host is not available, such as *P.

xanthii,* which can infect most of the cucurbit family (Miazzi *et al.,* 2011). Survival

also may occur in geographic regions favorable to both the pathogen and host, with

later dispersal of the pathogen to nearby regions as environmental conditions became

favorable there for disease development. Powdery mildew of wheat in China is an

example of this form of overwintering (Li *et al.,* 2013). Any combination of the above

will allow a powdery mildew pathogen to persist to the following season of its host.

The mode of perennation is influenced by climate and different modes can be used

within a species that is found in different climates (Jarvis *et al.,* 2002; Caffi *et al.,* 2013). Below is a more in-depth description of formation and importance of flag shoots and cleistothecia since these seem to be the most common modes of perennation.

Flag shoots

Green tissue on buds can be infected by powdery mildew fungi, allowing mycelium of the fungus to perennate asexually on dormant buds (Jarvis *et al.,* 2002). As infected buds emerge in the following season, associated shoots may be partially to entirely colonized by the fungus. The disease cycle is perpetuated by conidia produced on the affected shoot. These infected shoots are called flag shoots, so named because of their appearance as a white flag amongst otherwise green host tissue. A partial list of plants where powdery mildew flag shoots have been described include grape, apple, hop, gooseberry, currant, rose, maple, and dogwood. These infected shoots are important in the epidemiology of powdery mildews since flag shoots are generally the first appearance of mildew in a growing season and are important sources of secondary infections (Jarvis *et al.,* 2002). The first appearance of *P. macularis* is usually observed on diseased shoots in April or May (Royle, 1978), or earlier in the Pacific Northwestern U.S. This form of overwintering is especially important in the Pacific Northwest and in other systems where cleistothecia are not common or are not found, such as in apple powdery mildew (Spotts & Chen, 1984; Gent *et al.,* 2008).

The survival of infected buds may be influenced by temperature (Jarvis *et al.,* 2002; Spotts & Chen, 1984). In apple, Spotts and Chen (1984) demonstrated that mildew infected buds were more susceptible to freezing injury and death than healthy buds. When temperatures dropped below -22°C infected buds were likely to die whereas healthy buds could survive to temperatures dropping to -26°C. In hop, observations in the field and experiments using potted plants kept in varying environments show that following a mild winter or warmer temperatures there is an increased number of flag shoots in spring (Liyanage & Royle, 1976). It has also been speculated that temperature plays a role in *E. necator* production of flag shoots since flag shoots seem to be more common in regions with mild, Mediterranean climates (Gadoury & Pearson, 1988; Legler *et al.,* 2013).

The appearance of flag shoots from year-to-year can be predicted in some cases. In grape it is more common to find flag shoots on a plant or in an area of a field that was near a flag shoot the previous year (Sall & Wrysinski, 1981; Ypema & Gubler, 2000). Pearson and Gärtel (1985) found that most flag shoots were found on nodes 3 to 5 on first year canes of grape. It has been suggested that the buds of grape are infected by powdery mildew early in the growing season (Pearson & Gärtel, 1985; Ypema & Gubler, 2000). In apple, flag shoots generally arise from the terminal and two distal auxiliary buds, suggesting that the infection that leads to successful perennation occurs later in bud development (Butt, 1972). On apple, fungicide sprays regimes did not directly support later bud infection, but indicated early management in the season decreased the number of flag shoots (Butt, 1972; Clifford *et al.,* 1980). This

response could be indirect, though, and meditated by a chain of events leading to suppression of inoculum later in the season.

Cleistothecia

Cleistothecia are the teleomorph of powdery mildews. This sexual structure is generally thought to overwinter or oversummer the sexual spores, known as ascospores. Many species of powdery mildew fungi do not seem to form viable ascospores so the epidemiological significance of these spores is debatable (Yarwood, 1957), although lack of experimental evidence for functionality does not necessarily prove lack of biological function. In *Erysiphe necator*, osmotic pressure caused by free water causes cleistothecia to rupture and release ascospores with force (Gadoury & Pearson, 1990). In locations where *P. macularis* is found, such as in England or Germany, the pathogen may overwinter as cleistothecia. Signs of ascosporic infection associated with hop are thought to include discrete, white sporulating colonies of the fungus on leaves near the ground (Royle, 1978). Definitive evidence of ascospore infectivity in this host has been reported, though. Cleistothecia have not been observed in the Pacific Northwestern U.S. (Gent *et al.*, 2006; Ocamb *et al.*, 1999)

Cleistothecia normally appear towards the end of a host's growing season, which could be due to increased nutritional stress in the fungus (Jarvis *et al.,* 2002) or simply an increased likelihood of two compatible isolates coming in physical contact (Gadoury & Pearson, 1988). Cleistothecia start to form in sexual populations of *P. macularis* in July and continue to form throughout the season (Royle, 1978). This

could indicate that the hop powdery mildew fungus does not require low temperatures to initiate development of cleistothecia, as described for the closely related strawberry mildew pathogen *P. aphanis,* which requires a brief period of 13ºC or less to initiate formation of cleistothecia (Aslaf *et al.,* 2013). The formation of cleistothecia in some powdery mildews is known to suppress the formation of conidia (Gadoury *et al.,* 2010; Gadoury *et al.,* 2011; Aslaf *et al.,* 2013).

Sexual reproduction in powdery mildews generally is believed to be due to heterothallism (Jarvis *et al.,* 2002; Tollenaera & Laine, 2013; Wicker *et al.,* 2013). In heterothallic species a given isolate contains one of two mating-types idiomorphs and two isolates containing opposite mating types need to be present for plasmogamy to occur. Some of the powdery mildews described thus far to be heterothallic are *B. graminis* f. sp. *horei* and *B. graminis* f. sp. *tritici* (Brewer *et al.,* 2011), *E. necator* (Gadoury & Pearson, 1991; Brewer *et al.,* 2011), *P. aphanis* (Gadoury *et al.,* 2010), *P. xanthii* (Perez-Garcia *et al.,* 2009; Brewer *et al.,* 2011), *Podosphaera leucotricha* (Coyier, 1973), and *P. pannosa* (Bender & Coyier, 1985). Royle (1976) reported preliminary data that suggested that *P. macularis* is heterothallic. This may suggest that one reason cleistothecia are not observed in the Pacific Northwest is because of the presence of only one mating type in the region.

Only a few powdery mildews so far have been found to be homothallic. Homothallic species contain both mating types in one isolate, allowing self-fertility. So far, only *P. plantaginis* (affects natural population of *Plantago lanceolata*) has been shown to be homothallic in both mating assays and by molecular evidence

(Tollenaera & Laine, 2013). A few other mildews have been misreported to be homothallic in the past including *B. graminis* f. sp. *hordei* (Cherewick, 1944a) and *B. graminis* f. sp. *tritici* (Cherewick, 1944b), but later were shown to be heterothallic through mating assays and molecular characterization of mating type idiomorphs (Spanu *et al.,* 2010; Brewer *et al.,* 2011; Wicker *et al.,* 2013). Other mildews that have been described as both being homothallic and heterothallic depending on the host substrate, including *E. polygoni* (Smith, 1970) and *Sphaerotheca fuliginea* (Homma, 1933; Homma, 1937; McGrath, 1994; Bardin *et al.,* 2007). However, the mating type idiomorphs of *E. polygoni* and *S. fuliginea* have not been characterized. It is debatable whether the mating systems of these are dependent on host substrate or if variability in mating system is an artifact of contamination in one or more studies.

While homothallism in powdery mildews seems to be rare this could be due to the lack of characterized powdery mildew mating systems to date. While there are at least 900 species of powdery mildew fungi documented (Takamatsu, 2012), a review of literature for mating systems of powdery mildew by Tollenaera & Laine (2013) indicated that mating system has been characterized in only approximately 13 species. This deficiency most likely stems from the difficulties of working with obligate pathogens and lack of genetic resources for powdery mildew fungi, making such studies difficult (Brewer *et al.,* 2011; Tollenaera & Laine, 2013).

Development of Cleistothecia

In the early stages of development cleistothecia are a white translucent color, maturing to a light yellow or amber, and finally to a dark brown or black (e.g., Legler *et al.,* 2013). During this time the ascocarp also increases in size. Depending on the species, the final diameter of cleistothecia range from approximately 100µm to 200µm (Jarvis *et al.,* 2002). Although spherical in shape through development, at maturity cleistothecia are concave-convex (Jarvis *et al.,* 2002; Gadoury *et al.,* 2009). Liyanage and Royle (1976) demonstrated that *P. macularis* cleistothecia have a bimodal maturation, reaching maturity in both November and in March. The appendages of cleistothecia start to form early in development, when cleistothecia initials are approximately 20 µm in diameter in *P. aphanis* (Gadoury *et al.,* 2009), and differ in morphology depending on genus. Appendage morphology is a key diagnostic for identification to species level, which is complemented in modern taxonomy by ribosomal DNA and other sequence data (Braun *et al.,* 2002; Takamatsu, 2004).

The formation of cleistothecia is complex and has been described in detail by Luttrell (1951), Gordon (1966) and Braun (1987). It is thought that formation is similar in most species. In *E. necator,* cleistothecia development is initiated within 48 hours of hyphal contact of two compatible mating types (Gadoury & Pearson, 1988). The formation of cleistothecia begins when two uninucleate hyphae come in contact and one encircles the other (Gordon, 1966). The central cell receives a nucleus from the other surrounding hyphae and enlarges to some extent. This central cell is known as a pseudoascogonial cell and the surrounding hyphae is a pseudoantheridial cell, not an ascogonia cell or antheridial cell since they do not seem to play a direct role in

ascus formation (Gorgon, 1966). The fusion and establishment of a cytoplasmic

connection of these hyphae is known as plasmogamy, while the movement of the

nucleus is known as dikaryotization (Glawe, 2008). The pseudoanthridial and

pseudoascogonial cells divide to form the developing peridium (Gordon, 1966; Glawe,

2008). The peridium becomes darkly pigmented over time (Gordon, 1966).

Karyogamy occurs within certain binucleated cells above the middle part of the

centrum (Lutrell, 1951; Gordon, 1966). The cells that go through karyogamy enlarge

to form asci or a single ascus depending on the species (Gordon, 1966). Meiosis of the

fusion nucleus in developing asci is usually delayed until the centrum cells have been

absorbed, tending to be completed before the winter dormancy (Gordon, 1966; Braun,

1987). The number of ascospores per ascus differs among powdery mildew species,

ranging from two to eight (Braun, 1987).

Cleistothecia formation can be influenced by a variety of environmental and

host variables (Smith, 1970). One such variable is temperature. Some but not all

powdery mildews need a period of 13°C or less to initiate cleistothecia development,

such as *P. aphanis* (Gardoury *et al.,* 2010). Other powdery mildew fungi have no such

limitations and, if heterothallic, only need to be growing in close proximity to a

compatible mating type, as typified by *E. necator* (Gadoury & Pearson, 1988).

However, temperature does play a role in the development rate of cleistothecia, with

cooler temperatures delaying maturation, as shown in *E. necator* (Legler *et al.,* 2013).

The disease incidence in a given field can influence the occurrence of

cleistothecia in that field if both mating-types are present in the population. A greater

incidence of disease increases the likelihood of two mating types coming in contact with each other (Gadoury & Pearson, 1988). Greater levels of inoculum in one season increase the chance of formation of cleistothecia and generally lead to greater levels of primary inoculum the following season (Gadoury & Pearson, 1988; Legler *et al.,* 2013). Füzi (2003) found that with *E. necator* only 1% of leaves needed to be colonized in a vineyard to initiate the formation of ascocarps in Hungary.

The formation of cleistothecia can also be influenced by which mating types are found in a given population and when the mating types occur. There are reports of isolated populations of powdery mildew pathogens that only have one mating type present and due to their heterothallic nature are unable to sexually reproduce. Examples of this phenomenon include *P. xanthii* in southern Italy (Miazzi *et al.,* 2011) and certain isolated populations of *E. necator* (Montarry *et al.,* 2008).

Epidemiological Significance of Sexual Recombination

Sexual reproduction is usually thought to be important to both the origin and fitness of species. Sexual reproduction can impact virulence of certain fungi. For example, *Ustilago maydis*, a smut fungus, can only infect plants in its dikaryotic stage that is generated after mating (Feldbürgge *et al.,* 2004). It is also thought that certain genes required for sexual reproduction can be linked to virulence in some fungi, as exemplified in *Fusarium graminearum* (Zheng *et al.,* 2013).

Different reproductive strategies affect the genetic structure of species, including fungal pathogens. The genetic structure of a population is important in

determining the evolutionary rate of a pathogen and hence its adaptability to changing environments. Genetic structure and differentiation of populations also influence selection of management strategies in agricultural settings (Wolfe, 1984; Chen & McDonald, 1995; McDonald & Linde, 2002). Random mating (sexual) populations are expected to show higher degrees of genotypic diversity than those that are asexual. This has been shown to be true in *Puccinia graminis* f. sp. *tritici* in the Pacific Northwest, where the sexual stage occurs, versus asexual populations of the fungus in the Central Plains (Burdon & Roelfs, 1985). In *B. graminis*, primary infections resulting from sexual ascospores contained a greater number of more evenly distributed races than infections resulting from conidial (asexual) populations (Welz & Kranz, 1987).

Selection pressure in a pathogen population may be driven by host genotype and can be especially strong in an agricultural setting. In agricultural systems host genotype exerts a strong selection pressure for isolates of the pathogen with corresponding virulence factors, as seen with *B. graminis* f. sp. *hordei* in the late 1940s in Germany (Wolf & Schwarzbach, 1978). This selection can result in high levels of disequilibrium among particular combinations of these virulence factors (Wolfe & Knott, 1982; Chen & McDonald, 1995). This suggests that pathogen populations with high levels of asexual reproduction may have high levels of virulence factors associated with the most prevalent resistant gene(s) possessed by the host. In such situations, breeding strategies can be employed to limit recombination of virulence factors in the pathogen, for instance by deploying new combinations of

resistance genes (Chen & McDonald, 1995). However, in a pathogen population with high levels of sexual recombination this strategy is less effective because of increased levels of recombination in virulence factors in the pathogen. In this scenario, breeding strategies are better focused on race-nonspecific resistance because it is likely that mono or oligogenic resistance will be overcome quickly (Chen & McDonald, 1995).

Mating Type Locus in Ascomycetes

The mating type locus differentiates sexual dimorphism and regulates sexual reproduction in fungi. In heterothallic or self-incompatible Ascomycetes the mating type locus (*MAT1*) contains one of two different sequences located in the same chromosomal locus in their genome. Because these sequences are not obviously related by structure or common descent they are known as idiomorphs and not alleles (Debuchy & Turgeon, 2006). The standardized nomenclature for the idiomorphs is based on the conserved alpha1 (α1) domain motif found in the MATα1 transcription factor of *Saccharomyces cerevisiae*, with this idiomorph called *MAT1-1* and the corresponding gene being *MAT1-1-1*. The second idiomorph, dubbed *MAT1-2* (gene referred to as *MAT1-2-1)*, is characterized by a protein encoding a DNA-binding domain of the high mobility group (HMG) type. Exceptions to the standard nomenclature occur in *Neurospora sp.* and *Podospora sp*. because designations for the genes from these genera were established prior to the proposal to standardize nomenclature of mating type genes (Turgeon & Yoder, 2000).

In homothallic or self-compatible Ascomycetes both idiomorphs of the *MAT1* locus often are found in the same nucleus. Some species have complete copies of the genes while others contain truncated versions of the genes. Because both idiomorphs are in the same nucleus the nomenclature is slightly different in these organisms. In these situations, the locus is normally referred to as *MAT1/2*. The term idiomorph is inaccurate in these organisms since both idiomorphs are in the same haploid genome and there is not a second strain of opposite mating type (Turgeon & Yoder, 2000).

In Ascomycetes, the regions flanking the idiomorphs *MAT1-1* and *MAT1-2* are the conserved homologous genes *SLA2*, a cystoskeleton assembly gene and *APN2*, a DNA lyase gene (Debuchy & Turgeon, 2006). Idiomorphs of some species of Ascomycetes may contain additional genes in the region. For example some Ascomycetes contain in addition to *MAT1-1-1* a gene known as *MAT1-1-2*, which is characterized by an amphipathic α helix. These addition genes found with an idiomorph are assigned a number that corresponds to that of its homolog in other fungi that have a given MAT gene. If there is no homologous gene then the next sequential number is assigned (Turgeon & Yoder, 2000). The function of these additional genes is not well known.

In Leotiomycetes, the class to which powdery mildew fungi belong, *MAT1-1-4* is sometimes found in addition to *MAT1-1-1* (as in *Pyrenopeziza brassicae*). This gene encodes a metallothionein-like protein expressed during mating, although its role in the sexual cycle remains unknown. It has been proposed that the MAT1-1-4 protein scavenges metal ions that may be present at high level during the development of the

fruiting body on senescing plant tissue. *MAT1-1-3* is found in *Rhynchosporium secalis* and in some other species of Leotiomycetes. This gene is a fungal mating-type transcription factor belonging to the MATA_HMG box family. *MAT1-1-2* is also part of this family and is found in other Ascomycetes but has not yet been described in Leotiomycetes. Both of these genes are characterized by an HMG domain, similar to what is found in *MAT1-2-1* (Debuchy & Turgeon, 2006).

Mating Type Locus of Powdery Mildews

The first mating type idiomorph described in a powdery mildew fungus was the *MAT1-2* idiomorph of *B. graminis* f. sp. *hordei* (Spanu *et al.,* 2010). Since then, Brewer *et al.* (2011) described the structure of the mating type idiomorphs of *E. necator* and designed degenerate primers to help identify the mating type idiomorphs of other powdery mildews. Other structures of the *MAT1* loci described in powdery mildew organisms include *B. graminis* f. sp. *tritici* (Wicker *et al.,* 2013; Brewer *et al.,* 2011) and *P. plantaginis* (Tollenaera & Laine, 2013). None of the *MAT1-2* idiomorphs described thus far have had any other associated mating type genes. However, all organisms possessing the *MAT1-1-1* idiomorph also possess the *MAT1-1-3* gene but not the *MAT1-1-4* as found in some Leotiomycetes. The conserved regions of the mating idiomorphs (α box in *MAT1-1* and the HMG domain in *MAT1-2*) have been identified in other powdery mildew organisms, for instance *P. aphanis* (Asalf *et al.,* 2013), *P. xanthii* and *Microsphaera syringae* (Brewer *et al.,* 2011). The structure of the locus is unknown in these organisms.

Based on the few examples of powdery mildew fungi where the structure of *MAT1* is known, it appears that the genomic architecture in powdery mildew fungi is similar to one another but differs slightly from other Ascomycetes. For example, while homologous genes associated with the mating loci are found in powdery mildew organisms, they are not closely flanking the mating type locus (e.g., the *MAT1-2* idiomorph of *B. graminis* f. sp. *hordei*). In addition, the size of the *MAT1* locus of powdery mildew fungi appears to be much larger than that of other Ascomycetes. The distance between *SLA2* and *APN2*, the genes that typically flank the sides of *MAT1*, typically ranges between 3kb and 7kb with most Ascomycetes being around 5kb. In *B. graminis* the distance is approximately 117kb and in *E. necator* the distance is thought to be 10kb (Spanu *et al.,* 2010; Brewer *et al.,* 2011).

These differences may be due to the proliferation of transposable elements and repetitive sequences found in the genome of powdery mildew fungi (Spanu *et al.,* 2010; Brewer *et al.,* 2011; Tollenaera & Laine, 2013; Wicker *et al.,* 2013). Based on the *B. graminis* genomes available, powdery mildew genomes appear to be larger and more complex compared to other Ascomycetes. Wicker *et al.* (2013) classified 90% of the 180 megabase (MB) genome of *B. graminis* f. sp. *tritici* as transposable elements, making it the most highly-repetitive genome described in a fungus thus far. Other powdery mildew genomes (*B. graminis* f. sp. *hordei, E. pisi, Golovinomyces orontii*) range from 120 to 160 MB, making the size of the genomes more than four times larger than the median of other Ascomycetes (Spanu *et al.,* 2010). While the genomes are larger than most Ascomycetes, the number of genes ranges from approximately

5800 to 6500, which is relatively low compared to other Ascomycetes and probably reflects gene loss associated with biotrophic parasitism (Spanu *et al.*, 2000; Wicker *et al.*, 2013). Protein coding genes typically are found in small clusters interspersed between long stretches of transposable elements (Spanu *et al.*, 2010). Spanu *et al.* (2010) hypothesizes that the reason for the large number of transposable elements in the powdery mildews is because these organisms lack genes required for repeat-induced point mutations (RIPs), a genome defense mechanism in fungi, while still containing components necessary for mitotic and meiotic silencing. The lack of RIPs may also explain why the *MAT1-2* idiomorph is not flanked by the conserved genes found in other Ascomycetes (Spanu *et al.*, 2010; Wicker *et al.*, 2013).

Gaps in Knowledge of *Podosphaera macularis*

There is little known about the specific process of overwintering in *P. macularis*. The above ground parts of hop die back to ground level each year but the rootstock is perennial. Crown buds found at varying depths in the soil that are formed on modified stem material produce shoots in the spring. The upper most buds are usually dormant for approximately a year before breaking early in the growing season. Buds formed on rhizomes deeper in the soil can remain dormant for up to four years (Rybáček, 1991). While it is known that the fungus can overwinter in infected buds forming flag shoots the following spring, it is not known when these buds are susceptible and may become infected. Crown buds formed on new wood appear starting in July but are not reported

to have open scales that may be receptive to infection as in apple (Royle, 1978; Spotts & Chen, 1984).

The conditions favoring ascospore discharge and infection as well as temperatures favorable for the formation and maturation of cleistothecia are unknown for *P. macularis*. It has been thought that a wetting period caused by rain, dew, or irrigation is important for discharge and dispersal of ascospores as in other powdery mildew fungi (Jarvis *et al.*, 2002), although there are no studies supporting this assumption (Royle, 1978).

Liyanage (1973) attempted to propagate *P. macularis* on alternate hosts found around hop yards in England but was unable to identify an alternate host. The only confirmed alternate host of *P. macularis* is *H. japonicus*, a wild Japanese hop (Blodgett, 1913), but it appears that occurrence of *P. macularis* on *H. japonicus* has not been observed in a field setting. It is unclear whether *H. japonicus* or another alternative host can provide an alternate method of overwintering for *P. macularis*. *Cannabis* spp. are thought to possibly be an alternative host, as well as *Urtica* spp. However, host range studies have not been reported on either genus.

The population structure of *P. macularis* is largely unknown. Currently, virulence genes are differentiated in *P. macularis* by growing isolates on a differential set of hop cultivars containing different combinations of resistance genes. However, there is very little literature on the genetic basis of resistance of these cultivars and most of the information known about resistance in hop is uncharacterized. The different virulence genes leading to races of *P. macularis* are only postulated, not

published in primary literature, and literature definitively establishing differential hosts is possibly non-existent. Understanding the population structure and diversity of *P. macularis* in more detail could allow breeding programs to make more informed decisions on which cultivars to use as parents and overall breeding strategies. Determinants of pathogenicity and virulence of *P. macularis* at a molecular level are unknown. The obligate biotrophic lifecycle of powdery mildew fungi and complex genome structure make these systems intractable to molecular techniques employed with other fungi. Because of this, essentially no work investigating genome architecture and gene structure has been conducted in *P. macularis* or many other powdery mildew pathogens.

The research outlined in this thesis addresses a gap in knowledge known regarding cleistothecia initiation. This research establishes conclusively that *P. macularis* is heterothallic and the absence of cleistothecia in the Pacific Northwestern U.S. can be explained by the absence of the *MAT1-2* mating type. In addition, molecular tools for the identification of the mating type idiomorphs of *P. macularis* were developed and can potentially be used to help identify mating type loci in other members of the *Podosphaera* genus. Controlled environment studies were conducted which indicate that initiation of mating in *P. macularis* does not appear to require low temperature as found in related powdery mildew organisms. A description of the ontology of cleistothecia development by scanning electron microscopy is given. The functionality of ascospores of *P. macularis* is established for the first time.

CHAPTER 2:

Identification and distribution of the mating-type locus and development of

cleistothecia of *Podosphaera macularis*

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In progress

ABSTRACT

Podosphaera macularis, causal agent of hop powdery mildew, is known to produce

cleistothecia (syn. chasmothecia) in eastern North America and Europe, but ascocarps

are not reported from the Pacific Northwestern region of North America. Reasons for

the apparent absence of cleistothecia in the Pacific Northwest are unknown. We

established that *P. macularis* is heterothallic and that ascocarp ontogeny, maturation,

dehiscence, and ascospore infection proceeds similarly to other powdery mildew

fungi. Genome sequencing of a *MAT1-1* isolate revealed the structure of the *MAT1*

locus and presence of *MAT1-1-3* demonstrating further similarities to other powdery

mildew fungi. PCR assays with primers designed from conserved domains of the

MAT1 idiomorphs were developed to characterise the frequency of idiomorphs in

populations of *P. macularis.* Amongst 317 samples of *P. macularis* collected during

2012 and 2013 from the Pacific Northwest only the *MAT1-1* idiomorph was found. In

contrast, among 56 isolates from the eastern United States and Europe, *MAT1-1* and

MAT1-2 idiomorphs were detected at equivalent frequencies. At temperatures

representative of late season conditions in the Pacific Northwest, cleistothecia formed

readily when a *MAT1-1* isolate from the Pacific Northwest was paired with a *MAT1-2*

isolate collected from outside the region. Although these findings do not encompass

all climatic, geographic, or temporal barriers that could inhibit the formation of

cleistothecia, the current absence of the ascigerious stage of *P. macularis* in the Pacific

Northwest could be explained by the absence of the *MAT1-2* mating type idiomorph.

Introduction

Hop powdery mildew, causal agent *Podosphaera macularis,* is one of the oldest and economically important fungal diseases of hop (Neve, 1991). If left unmanaged powdery mildew is capable of causing complete crop loss and was partially responsible for the demise of the hop industry in New York State in the early 1900's (Blodgett, 1913; Barth *et al.*, 1994). Despite the hop industry being present in the Pacific Northwestern region of North America since the 1900s (Barth *et al.*, 1994), powdery mildew was not reported in hop yards of the Pacific Northwest until 1997 (Ocamb *et al.*, 1999). While the ascigerious stage of *P. macularis* is known to exist in Europe (Neve, 1991) and eastern North America (Blodgett, 1913), the ascigerious stage has not been reported in the Pacific Northwest (Gent *et al.*, 2006).

Where the sexuality of powdery mildew fungi has been experimentally determined, most species have been found to be heterothallic (Jarvis *et al.*, 2002; Tollenaera & Laine, 2013; Wicker *et al.*, 2013; Gadoury & Pearson, 1991; Gadoury *et al*., 2010). Homothallism in powdery mildew fungi has been reported in relatively few cases (Homma, 1933; Smith 1970), and some of these same species have also been described as heterothallic (Gadoury & Pearson, 1991). Only in *Podosphaera plantaginis* has homothallism been confirmed by mating assays and molecular evidence (Tollenaera & Laine, 2013). The relative rarity of homothallism in the powdery mildew mating systems might reflect that relatively few systems have been

experimentally investigated. Nonetheless, heterothallism appears to be more common among powdery mildews than homothallism.

Sexual recombination can be important for a pathogen's ability to adapt to changing environments and hosts, survival of unfavorable environmental conditions, and development of resistance to fungicides. In some fungi a sexual resting structure can aid in survival overwinter or during the absence of a host (McDonald & Linde, 2002; Agrios, 2005). The need for survival apart from the host during the intercrop period is especially acute in the case of a biotrophic pathogen such as *P. macularis*. The lack of the ascigerious stage of *P. macularis* in the Pacific Northwest forces the pathogen to overwinter vegetatively in association with crown buds of dormant hop plants or on hop plants cultivated in greenhouses (Gent *et al.*, 2008). Understanding why cleistothecia do not form in this population is important because the mode of perennation has important implications for management practices of hop, such as pruning in the late spring, and sexual reproduction that might allow for additional mechanisms for survival and genetic adaptation.

In most heterothallic filamentous ascomycetes, the *MAT1* locus controls mating. The *MAT1* locus contains one of two different sequences, *MAT1-1 or MAT1-2*. Because these sequences are not obviously related by structure or common descent they are known as idiomorphs and not alleles (Debuchy & Turgeon, 2006). The *MAT1-1* idiomorph is characterized by the *MAT1-1-1* gene that encodes a protein containing an alpha 1 (α1) box, while *MAT1-2* is characterized by the *MAT1-2-1* gene

that encodes a protein containing a high mobility group (HMG) domain (Turgeon &

Yoder, 2000). Idiomorphs may also contain other mating type related genes, such as

MAT1-1-3, which is found in addition to *MAT1-1-1* in some species of ascomycetes

(Debuchy & Turgeon, 2006) as well as the powdery mildew fungi *Erysiphe necator*

(Brewer *et al.*, 2011), *Blumeria graminis* f. sp. *tritici* (Wicker *et al.*, 2013), and

Podosphaera plantaginis (Tollenaera & Laine, 2013). The regions flanking mating

type idiomorphs are homologous for both mating types and the gene order in the

flanking regions is conserved amongst most ascomycetes (Debuchy & Turgeon,

2006), but appears slightly different in the powdery mildew pathogens described thus

far (Brewer *et al.*, 2011; Spanu *et al.*, 2010; Wicker *et al.*, 2013). The unique

architecture of the *MAT1* locus in powdery mildews could be related to the high levels

of transposable elements found in these genomes. For instance, in *B. graminis* f. sp.

tritici, over 90% of the genome was classified as transposable elements (Wicker *et al.*,

2013).

With the creation of degenerate primers to identify the conserved regions of

the *MAT1* locus in the Erysiphales (Brewer *et al.*, 2011) there is an opportunity for

expanding the body of knowledge on mating systems in powdery mildew fungi. A

thorough evaluation of mating system and characterization of ascocarp initiation and

development has not been conducted in *P. macularis*, leading to speculation on the

basis for the absence of the ascigerious stage of *P. macularis* in the Pacific Northwest.

Royle (1976) stated that *P. macularis* was heterothallic, and it has been assumed that

the lack of the ascigerious stage is due to absence of one of the necessary mating types

needed for sexual reproduction. Other possibilities that could explain the absence of cleistothecia are various climatic, geographic, or temporal barriers that prevent sexual reproduction. This research sought to clarify if and why the sexual stage of the pathogen is absent from the Pacific Northwest. This was accomplished by characterizing the mating type idiomorphs, developing PCR assays for rapid determination of mating type, and conducting a survey for mating type idiomorphs among populations in the Pacific Northwest and within populations in eastern North America and Europe where cleistothecia are known to occur. Physical pairings of the fungus were also conducted under three different temperature regimes to determine if incubation temperature might affect the initiation of cleistothecia, as had been reported for the closely-related pathogen *Podosphaera aphanis* (Aslaf *et al.,* 2013).

Materials and Methods

Plant materials

Plants of the powdery mildew susceptible cultivar Symphony were propagated from softwood cuttings and maintained in a greenhouse free of powdery mildew. The greenhouse was maintained at 20 to 25ºC with a 14-h photoperiod. Plants were grown in Sunshine Mix #1 (SunGro Horticulture) for approximately 21 days and were watered regularly receiving Sunshine Technigro 16-17-17 Plus fertilizer with micronutrients (Sun Gro Horticulture) at each irrigation to promote succulent and vigorous growth. Young, unfurled leaves from the top node were detached and used to maintain cultures of *P. macularis*.

Discovering the *MAT1* locus in *P. macularis*

Degenerate primers designed by Brewer *et al.* (2011) for the mating type idiomorphs

of powdery mildew fungi were used to identify the conserved region of the *MAT1-2*

idiomorph (genbank accession KJ741396) of *P. macularis* using DNA obtained from a

field population of the pathogen from the Czech Republic. The PCR reaction was

carried out in a total volume of 12.5 μl. Reaction components and cycling parameters

were as described by Brewer *et al.* (2011) for degenerate primers except reaction

components and volume were halved. Ten microliters of the PCR product was

visualized by electrophoresis through a 1% (w/v) agarose tris-acetate-EDTA gel. The

approximately 230-bp PCR product was purified from the gel (UltraClean GelSpin

DNA Extraction Kit, Mobio) and cloned using a TOPO TA cloning kit (Invitrogen).

The cloned PCR products were sequenced bi-directionally at the Oregon State

University Center for Genome Research and Biocomputing.

The degenerate primers designed for the conserved region of *MAT1-1* were

unsuccessful at identifying the *MAT1-1* idiomorph in *P. macularis* despite multiple

attempts using DNA originating from the Pacific Northwest and from the Czech

Republic. *MAT1-1* of *P. macularis* was identified using primers Pxα-10f and Pxα-

156r, designed for the closely related species *P. aphanis* (Asalf *et al.*, 2013). The PCR

reaction was carried out in a total volume of 12.5 μl with reaction components as

stated above. Cycling conditions included an initial denaturation at 95°C for 2 min

followed by 35 cycles with a denaturation step at 95°C for 30 s, annealing set as a

gradient from 48 to 58°C for 30 s, extension at 72°C for 30 s, followed by a final

extension at 72°C for 5 min. The approximately 170-bp PCR product was gel purified, cloned, and sequenced as described previously.

PCR developed for *P. macularis*

Specific primers incorporating single nucleotide polymorphisms from the closely related organisms *P. xanthii* and *P. aphanis* were designed from the conserved region of *MAT1-2* of *P. macularis*. The primers sequences were M2F_2_TA (5-GTCTTAGCAATAATCAAATATG) and M2R_2_Edeg (5-TTCCGTGGTTTGTAACGGTAA). Because of the limited size of the conserved region for *MAT1-1*, the primers from Asalf *et al.* (2013) were used in this study. The PCR reaction for both primer sets was carried out identically in a total volume of 25μl. Reaction components included: 10μl of Mango Taq (Bioline), 0.5μl of 50% acetonitrile in water, 0.75μl of each 10μM primers, and 1.0μl of DNA template. Cycling conditions included an initial denaturation at 95°C for 2 min, followed by 35 cycles with a denaturation step at 95°C for 1min, annealing at 52°C for 1 min, and extension at 72°C for 30 s. The final extension was at 72°C for 10 min.

Verification of primers for *P. macularis*

Clonal isolates of *P. macularis* were tested with the above primers. Tester isolates were selected for *MAT1-2* and *MAT1-1* originating from Europe. Clonal isolates from the Pacific Northwest, 15 in total, were paired individually with both testers on detached leaves for approximately 21 days at 13°C with a 14 h photoperiod. Isolates

were also paired with themselves as a check of whether the fungus is capable of self-mating. The presence or absence of cleistothecia was confirmed 21 days after inoculation with the aid of a stereomicroscope at 10 to 30X magnification.

Specificity and sensitivity of primer sets

Attempts at amplification of other organisms were made with both the *MAT1-1* and *MAT1-2* primer sets to determine the specificity of the primers. Organisms tested were various other powdery mildew fungi found on grape, cucumber, zucchini, maple, strawberry, prostrate knotweed, plantain weed, rose, Oregon grape, heavenly bamboo, azalea, grass species, cherry, lilac, blackberry, buckeye, and sycamore. Also tested were organisms commonly found in association with powdery mildew fungi, namely *Lecanicillium attenuatum* and *Pseudozyma flocculosa*, and organisms found in association with hop, *Cladosporium herbarum, Fusarium* spp., *Epicoccum nigrum,* an *Alternaria* spp., and *Pseudoperonospora humuli*, as well as hop itself.

The ability of the primer sets and the DNA extraction method to detect varying quantities of *P. macularis* was tested. DNA was extracted from powdery mildew isolates using a chelex extraction procedure (Brewer & Milgroom, 2010). The reliability of the assay and extraction procedure was established by collecting conidia and mycelia of the fungus on to cellophane tape and extracting DNA from pieces of tape of varying sizes. The standard size of tape normally used was 2.5 cm in length, and ¾, ½, ¼, and $^1/_8$ of the standard length were tested. This was repeated independently 9 times. Extracted DNA was then tested with the PCR assays to

determine the proportion of PCR assays that successfully amplified *P. macularis* DNA.

Determining the full length of the *MAT1-1* idiomorph

The *MAT1-1* idiomorph of an isolate of *P. macularis* originating from Oregon was sequenced using paired-end 100 bp Illumina HiSeq 2000 technology at the Oregon State University Center for Genomics Research and Biocomputing. De novo assembly was achieved using Velvet 1.2.09 (Zerbino & Birney, 2008) with a minimum contig length of 200 bp required for retention. Multiple initial assemblies were performed using 4 to 60 million reads and a k-mer length ranging from 31 to 61 bp. After all initial assemblies had been completed the contigs from each assembly were clustered into groups of homologous sequences using Vmatch 2.0 (Abouelhoda *et al.,* 2004). The longest sequence was retained from each cluster. These sequences, along with the sequences that did not cluster with other sequences, were concatenated for the final assembly. *MAT1-1* and related genes were isolated from assembled contigs using Geneious version 6.0.5 (Biomatters) using available gene information of other powdery mildew fungi on the National Center for Biotechnology Information GenBank.

To join the gene *SLA2* with *MAT1-1* a PCR assay was designed to amplify the region between the two genes. Primers utilized were PM_SLA2-mat1F2 (5-TGTAGCTATCTAGATTCGTCCAT) and PM_SLA2-mat1R2 (5-TGAAAGGATCTCGGTTCCAC). The PCR reaction components and cycling

conditions were the same as stated above for the mating type PCR assay with the exception of the annealing temperature being set at 60°C. The approximately 700-bp PCR product was gel purified, and sequenced bidirectionally by the Center for Genomics Research and Biocomputing.

Mating type survey

A stratified sampling method was used to collect samples of powdery mildew from the Pacific Northwest for determination of mating type. Sampling occurred three times during each of 2012 and 2013: early season (early June 2012, early May 2013), mid season (mid July 2012, mid June 2013), and late season (early September 2012, mid July 2013). At least 3 hop yards were sampled from each of Oregon and Washington at each time point. Powdery mildew samples also were collected from Idaho during the late collection in 2012, but were not available from Idaho during the other surveys because powdery mildew was not reported. When possible, different cultivars of hop were selected to represent the diversity of cultivars grown in the Pacific Northwest. Powdery mildew samples from the following cultivars were tested over the course of the survey; Apollo, Bravo, Cascade, Centennial, Chelan, Chinook, Cluster, Columbus/Tomahawk, El Dorado, Galena, Glacier, Magnum, Meridian, Millennium, Mt. Hood, Nugget, Santiam, Sterling, Summit, Super Galena, Symphony, Tettnanger, Warrior, Willamette, Zeus and various unnamed breeding lines. Four to five samples from separate leaves and/or cones were collected from each field during each sampling period. In addition, a total of 56 samples of hop leaves with powdery mildew

or clonal isolates of *P. macularis* from known sexual reproducing populations were obtained from cooperating scientists in the eastern U.S. and Europe. When samples were received in a viable condition, *P. macularis* was cultured and maintained on detached hop leaves before DNA extraction. Otherwise, 2mm disks of infected tissue were extracted using a PowerSoil DNA isolation kit (Mo Bio Laboratories). Template DNA was utilized in PCR assays as described above for detection of *MAT1-1* and *MAT1-2*. Negative and positive controls were included in every PCR assay using DNA extracted from confirmed *MAT1-1* and *MAT1-2* isolates. In 2012, the ITS region also was amplified using ITS primers ITS1 and ITS4 (White *et al.*, 1990) as a further control to ensure amplification of the template DNA was possible. To further guarantee that the products of the PCR reactions were obtained from *P. macularis*, every PCR product was confirmed by bi-directional sequencing in 2012. In 2013, a subsample of PCR products obtained were verified by sequencing. Sequences were aligned to each other and to *P. aphanis* and *P. xanthii* using BioEdit (Hall, 1999) to verify that SNPs associated with other powdery mildew fungi were not present.

Effects of temperature on initiation of cleistothecia

To determine if temperatures typical of the growing season for hop in the Pacific Northwest might suppress initiation of cleistothecia (Asalf *et al.,* 2013), compatible isolates were paired on mildew-free leaves as described previously. The inoculated leaves were incubated in a diurnal temperature regime in which hourly temperatures ranged from 11°C to 27.9°C (Table 4). Values for the hourly temperatures were

derived from the 30-year average daily temperatures for Prosser, Washington USA for the date 1 September as described by De Wit *et al.* (1978). Weather data was retrieved from the Western Region Climate Center (Prosser, WA USA). A second group of paired isolates was also incubated on detached leaves at a constant temperature of 13°C (Table 4), while a third group was incubated at ambient room temperature, which ranged from 16°C to 26°C over the course of the experiment (Table 4). All treatments were replicated on 9 leaves, and the experiment was conducted three times. At 28 days after inoculation, leaves were examined for the presence of cleistothecia within powdery mildew colonies. Differences among means of colonies forming cleistothecia at different temperature regimes were analysed in a generalized linear mixed model with the response distribution specified as the beta distribution and a logit link function using the GLIMMIX procedure in SAS version 9.4 (SAS Institute).

Maturation process of cleistothecia and viability of ascospores

Leaves bearing cleistothecia in various maturation stages were gathered from feral hop plants near Sharpsburg, Maryland in October of 2012. Disks approximately 2mm in diameter with cleistothecia present were cut from the leaves. Disks for scanning electron microscopy were fixed in 3% glutaraldehyde in a water solution overnight. Samples were then dehydrated in a five step ethanol series, critical point dried, mounted, and sputter coated with gold before examination. Samples intended for light microscopy were fixed in formalin/acetic acid/alcohol (Johansen, 1940) and dehydrated in an ethanol series, infiltrated with paraffin, and sectioned at 10 to 15 μm.

The sections were adhered to glass microscope slides, dewaxed in Histoclear, and stained with 1% safranin in 50% ethanol, followed by counterstaining in 0.1% fast green in clove oil (Johansen, 1940). Samples were examined under light microscope at 10 to 40X magnification.

To check viability of ascospores, cleistothecia were mounted on glass microscope slide in a 0.5% (wt/vol) fluorescein diacetate (FDA). The wall of the cleistothecium was ruptured by applying gentle pressure to the glass coverslip using fine forceps as the ascocarp was observed at 100X magnification. The ascus contents were then allowed to absorb the FDA stain for 5 minutes before viewing and photomicrography at 200X magnification under both bright-field and fluorescence illumination (325 to 500 nm excitation filter and transmission filter >530 nm). Cleistothecia prepared as above were also stained with Sudan Black B and examined at 200X magnification under brightfield illumination to assess the lipid content of the ascus and ascospores (Gadoury *et al.,* 2010), as reabsorbtion and metabolism of lipids has been correlated with maturation of ascocarps in *P. aphanis* (Gadoury *et al.,* 2010) and *Erysiphe necator* (Gadoury & Pearson, 1990).

To further functionality and infectivity of ascospores, detached leaves bearing cleistothecia were collected in September 2013 from a feral hop plant in Seneca Castle, New York, placed in envelopes made from fiberglass window screen, and allowed to overwinter on the ground in Geneva, NY. At monthly intervals from January to March and at 2-week intervals thereafter, 2-cm disks bearing at least 20 ascocarps were cut from the leaves, attached to a 9-cm disk of filter paper wetted with

distilled water, and suspended above mildew-free detached hop leaves of the cultivar

Symphony for 24 h at 20 to 22°C. After exposure to cleistothecia, detached leaves

were enclosed in 9-cm polystyrene Petri dishes and incubated at 20 to 22°C for 14

days. Leaves were then examined at 20X magnification for the presence of mildew

colonies.

Results

Verification of primers for *P. macularis*

PCR products of the expected α1 box and HMG domains were obtained with the

MAT1-1 and *MAT1-2* tester isolates originating from Europe. Physical pairings

conducted with an isolate of *P. macularis* from the Pacific Northwest and isolates

identified as *MAT1-1* and *MAT1-2* genotypes by PCR were consistent (Table 1).

Cleistothecia developed only when isolates of opposite mating types were paired

together and were not produced when two Pacific Northwest isolates were paired.

Using the tester isolates, 15 additional pairings were conducted with clonal isolates

from the Pacific Northwest that were all positive for *MAT1-1* by PCR with the same

outcome.

Specificity and sensitivity of primer sets

The *MAT1-1* primers cross-reacted with DNA from the powdery mildew fungi on

strawberry, blackberry, and rose. The *MAT1-2* primers cross-reacted with the powdery

mildew fungus growing on cherry. The PCR assays did not cross-react with any other

organism tested. Both the *MAT1-1* and *MAT1-2* primer sets strongly amplified a DNA product in every experiment when using only $^1/_8$ of the quantity of material normally used in the extraction procedure.

Determining the full length of the *MAT1-1* idiomorph

One lane of paired-end 101 bp Illumina HiSeq 2000 sequencing resulted in 210,557,723 pairs of reads. The assembly of a subset of these reads resulted in 347,507 contigs ranging in size from 200 bp (the lower threshold) to 16,809 bp in length. The reference included a total of 126,225,625 nucleotides with an N50 of 329 bp. A single contig of approximately 2,200bp was isolated containing the full length of the *MAT1-1-1* gene (genbank accession KJ922755) of *P. macularis* found in the *MAT1-1* idiomorph. In addition, fragments of genes associated with the *MAT1-1* idiomorph, *SLA2* (genbank accession KJ922754) and *APN2* (genbank accession KJ830621) were found in the sequence database along with *MAT1-1-3* (genbank accession KJ830622) but were unable to be joined to *MAT1-1-1* since all were on separate contigs. However, the contig containing *MAT1-1-1* and the contig containing *SLA2* were joined later by a PCR amplifying across the missing region between contigs and producing the full-length sequence of *MAT1-1-1*.

Mating type survey

   A total of 183 and 134 hop samples bearing colonies of *P. macularis* were collected from the Pacific Northwest in 2012 and 2013, respectively (Table 2). Only the *MAT1-*

1 idiomorph was found. The 99% confidence interval for a binomial test of proportions was 0.98 to 1, providing strong evidence that *MAT1-2* is not present or prevalent in the Pacific Northwest region. The mating types of *P. macularis* isolates on 56 samples collected within populations outside of the Pacific Northwest were identified. Of these 56 samples collected, a total of 38 tested positive for *MAT1-1* and a total of 44 tested positive for *MAT1-2,* i.e., *MAT1-1* and *MAT1-2* were detected in the same sample multiple times in these populations (Table 3). The ratio of idiomorphs detected was consistent with both mating types being distributed in a ratio approximating 1:1 in both North America (Chi square test *P* = 0.508) and Europe (Chi square test *P* = 0.841), with a combined ratio also similar to 1:1 (Chi square test *P* = 0.508).

Effects of temperature on initiation of cleistothecia

Irrespective of temperature treatments, from 38 to 52% of the leaves inoculated with compatible isolates formed abundant cleistothecia within 28 days after inoculation (Table 4). The incidence of ascocarp initiation did not differ significantly (*P* = 0.05) between leaves incubated at a constant temperature 13°C compared to the diurnal temperature cycle (Table 4), and was only slightly elevated at ambient room temperatures ranging from 16 to 26°C (Table 4).

Maturation process of cleistothecia and viability of ascospores

Appendages typical of the myceloid appendages for the genus *Podosphaera* began to

appear once ascocarps had reached a diameter of approximately 15 to 20 µm (Fig. 1).

These appendages continued to grow as the ascocarp increased in diameter and

eventually grew down and became entangled in the pannose mycelium of the mildew

colony, effectively anchoring the ascocarp to the developing mildew colony. By the

time the ascocarp was 40 to 50 µm in size the ascocarp wall had changed from hyaline

to yellow, most likely due to the accumulation of a yellow lipid compound that was

darkly stained by Sudan Black B (Fig. 2). At 80 to 90 µm in diameter the ascocarp

wall was composed of thick-walled dark cells and a single ascus containing eight

ascospores was present (Fig. 2). Ascospores fluoresced when stained with FDA (Fig.

2). As this process occurred, ascocarps and appendages became further entangled to

the mildew colony and thus host tissue due to overgrowth by pannose mycelium (Fig.

1). After overwintering, rupture of the ascus forcing ascospore release generally

occurred within 5 min of dehiscence at 22 to 25°C (i.e., room temperature) so long as

the ascocarp remained in contact with a film of water. Resulting colonies from

ascospore infection could be seen after approximately 14 days of incubation (Fig. 2).

Discussion

Our results indicate that the absence of the ascigerious state of *P. macularis* in

the Pacific Northwest region is due to the exclusive presence of only one mating type:

MAT1-1. We tested 317 isolates over two seasons and all were positively identified as

MAT1-1. In other regions where ascocarps are known to occur, we were able to detect

MAT1-1 and *MAT1-2* at approximately equal frequencies. Isolates of *MAT1-1*

collected within the Pacific Northwest region readily formed ascocarps when paired with *MAT1-2* across a range of temperatures that would include much of the growing season in the region. Thus, three lines of evidence (assays using specific primers, crosses of isolates, and controlled environment studies) converge to support the conclusion that only *MAT1-1* is currently present in the region.

It is often the case that establishment of a pathogen in a new region is the result of only one or a few isolates of the pathogen being introduced, resulting in a genetic founder effects. If this new, derived population remains isolated from the original population and has only a small subset of genetic variation found in the larger original population, then this founder effect may persist, especially in clonal organisms as observed in certain populations of oomycetes, ascomycetes, and basidiomycetes. Understanding how and when sexual reproduction occurs in a population and modes of perennation for a pathogen are important in disease management (Grünwald & Goss, 2011; McDonald & Linde, 2002). This is especially true for pathogenic foliar fungi given the capability of long distance dispersal by wind, allowing the pathogen to establish and re-establish in multiple regions (Brown & Hovmoller, 2002).

Mating assays verify the observation of Royle (1976) that *P. macularis* is heterothallic. Absence of the *MAT1-2* idiomorph of *P. macularis* in the Pacific Northwest forces the pathogen to survive the winter in association with living host tissue. The presence of only *MAT1-1* in the Pacific Northwest may be the result of an isolated introduction of *P. macularis* to the region during the mid-1990s, either consisting only of strains of the *MAT1-1* idiomorph or perhaps both idiomorphs, with

the *MAT1-2* type strains being unsuccessful at establishing or surviving for unknown reasons. The absence of sexual recombination forces *P. macularis* through a severe bottleneck event each winter, as flag shoots are produced infrequently in commercial hop yards (Gent *et al.*, 2008). Because the ascigerous stage the pathogen is absent, *P. macularis* is controlled currently in the Pacific Northwest through spring pruning of hop plants to destroy the emerging flag shoots, either by mechanical or chemical means (Gent *et al*., 2012). The absence of ascosporic inoculum in the Pacific Northwest greatly simplifies the management program compared to other regions where both flag shoots and ascosporic inoculum may occur.

Recently a new strain of *P. macularis* capable of overcoming a highly deployed resistance gene in hop, R6, has been observed in the Pacific Northwest (Wolfenbarger *et al.,* 2013). Sampling in this study of *P. macularis* from cultivars possessing R6 verified that this virulent strain is only present as *MAT1-1*, suggesting that the new strain is either an extant but rare *MAT1-1* isolate that was selected through widespread deployment of the R6 resistant gene, a spontaneous mutant of an extant *MAT1-1* isolate that was thereafter selected by the R6 host gene, or a newly-introduce *MAT1-1* isolate of *P. macularis* from another region. The latter seems unlikely given that mating type idiomorphs occur in approximately equal ratios outside of the Pacific Northwest.

Some powdery mildew fungi such as *P. aphanis* require brief periods of low temperature to initiate ascocarps (Asalf *et al.*, 2013). *P. macularis* appears to lack a strict temperature requirement for initiation of ascocarps, readily forming cleistothecia

at all temperature regimes evaluated, in addition to constant 18°C. This provides further evidence to support the hypothesis that *MAT1-2* is absent in the Pacific Northwest since the temperatures found during late season appear to be conducive for ascocarp formation, implying if both mating types were present then cleistothecia could be formed readily.

The maturation process of cleistothecia formed by *P. macularis* was documented by means of microscopy and vital staining. The maturation process of *P. macularis* appears very similar to that of *P. aphanis* (Gadoury *et al.*, 2010) and other powdery mildew fungi (Jarvis *et al.*, 2002). Colonies resulting from ascosporic infection were observed in laboratory experiments. Liyanage & Royle (1976) described powdery mildew on hop early in the growing season that appeared to be the result of ascospore infection based on circumstantial evidence. However, their attempts to demonstrate ascospore infectivity experimentally failed. The present study is, to our knowledge, the first documentation of ascospore viability and infectivity in *P. macularis*.

There is limited molecular information on mating type systems of powdery mildew fungi. This is most likely because of the obligate nature of these organisms, which makes them difficult to work with. In an attempt to better understand the architecture of the *MAT1* locus in *P. macularis* and to create a molecular database for future studies, genomic sequencing was conducted on a *MAT1-1* isolate originating from Oregon. In other ascomycetes, there is a conserved structure of the *MAT1* locus where the mating type idiomorph and related genes are flanked by the genes *APN2*

and *SLA2*. While the *MAT1-1-1* gene in *E. necator* (Brewer *et al.*, 2011) and *Blumeria graminis* f. sp. *tritici* (Wicker *et al.*, 2013) are flanked by the gene *SLA2, MAT1-1-1* genes in these organisms could not be joined to *APN2*. As found in these other powdery mildew fungi, the *MAT1-1-1* gene of *P. macularis* is flanked by the *SLA2* gene but could not be connected to *APN2*, despite *APN2* being present in the genome. The *MAT1-1-3* gene was also detected in the genome of *P. macularis* but was unable to be connected to the *MAT1-1-1* gene, similar to other powdery mildews (Brewer *et al.*, 2011; Wicker *et al.*, 2013).

The molecular characterization of mating type idiomorphs conducted in this research extends knowledge and genetic resources available for the genus *Podosphaera* and potentially other genera of Erysiphaceae. With the confirmation that this population is presently surviving the intercrop period asexually, this system could be a model for population genetic studies of asexually reproducing populations cycled through annual bottleneck events. In addition, the research presented here has provided information that has resulted in changes in quarantine regulations by the state departments of agriculture in Washington, Oregon, and Idaho to reduce the likelihood of the introduction of the second mating type of *P. macularis*. It is thought that the presence of the second mating type and the ascigerious stage would substantially increase seasonal carryover of inoculum and decrease the effectiveness of current management strategies for powdery mildew (Gent *et al.*, 2012). Long term, the presence of the ascigerious stage would undoubtedly change the diversity of the population of *P. macularis* found in the Pacific Northwest.

Acknowledgements

References

Abouelhoda M, Kurtz S, Ohlebusch E, 2004. Replacing suffix trees with enhanced suffix arrays. *Journal of Discrete Algorithms* **2**, 53–86.

Agrios, GN, 2005. *Plant Pathology*, 5th *edition*. San Diego, California: Elsevier-Academic Press.

Aslaf B, Gadoury DM, Tronsmo AM, Seem RC, Cadle-Davidson L, Brewer MT, Stensvand A, 2013. Temperature regulates the initiation of chasmothecia in powdery mildew of strawberry. *Phytopathology* **103**, 717-24.

Barth HJ, Klinke C, Schmidt C, 1994. *The Hop Atlas*. Nuremberg, Germany: Joh Barth. and Sohn.

Blogett FM, 1913. Hop Mildew. Bulletin Cornell University Agricultural Experiment Station **328**, 278- 310.

Brewer MT, Milgroom MG, 2010. Phylogeography and population structure of the grape powdery mildew fungus, *Erysiphe necator,* from diverse *Vitis* species. *BMC Evolutionary Biology* **10**, 268.

Brewer MT, Cadle-Davidson L, Cortesi P, Spanu PD, Milgroom MG, 2011. Identification and structure of the mating-type locus and development of PCR-based markers for mating type in powdery mildew fungi. *Fungal Genetics Biology* **48**, 704-13.

Brown JKM, Hovmoller MS, 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297**, 537- 41.

Bushnell WR, 2002. The role of powdery mildew research in understanding host-parasite interaction: past, present, and future. In: Bélanger R, Bushnell WR, Did AJ, and Carver TLW, eds. *The Powdery Mildews, A Comprehensive Treatise*. St. Paul, MN: The American Phytopathological Society, 1-12.

Darby P. 2013. Could there be a dominant gene for susceptibility to hop powdery mildew? *International Hop Growers Convention*,17-20.

Debuchy R,Turgeon BG, 2006. Mating-type structure, evolution, and function in Euascomycetes, In: Kües U, Fisher R eds. *The Mycota vol I. Growth, Differentiation and Sexuality*, Berlin, Germany: Springer Berlin Heidelberg, 293-323.

De Wit CT, Goudriaan J, van Laar HH, Penning de Vries FWT, Rabbinge R, van Keulen H, Sibma L, de Jonge C, 1978. Simulation of assimilation, respiration and transpiration of crops. Pudoc, Wageningen, The Netherlands: Simulation Monographs. pp 141.

Gadoury DM, Pearson RC, 1988. Initiation, development, dispersal, and survival of cleistothecia *Uncinula necator* in New York vineyards. *Phytopathology* **78**, 1413-21.

Gadoury DM, Pearson RC, 1990. Ascocarp dehiscence and ascospore discharge in *Uncinula necator*. *Phytopathology* **80**, 893-901.

Gadoury DM, Pearson RC, 1991. Heterothallism and pathogenic specialization in *Uncinula necator*. *Phytopathology* **81**,1287-93.

Gadoury DM, Asalf B, Heidenreich MC, Herrero ML, Welser MJ, Seem RC, Tronsmo AM, Stensvand A, 2010. Initiation, development, and survival of cleistothecia of *Podosphaera aphanis* and their role in the epidemiology of strawberry powdery mildew. *Phytopathology* **100**, 246-51.

Gent DH, Mahaffee WF, Turechek WW, 2006. Spatial heterogeneity of the incidence of powdery mildew on hop cones. *Plant Disease* **90,** 1433-40.

Gent DH, Nelson ME, George AE, Grove GG, Mahaffee WF, Ocamb CM, Barbour JD, Peetz A, Turechek WW. 2008. A decade of hop powdery mildew in the pacific northwest. Online. *Plant Health Progress* doi:10.1094/PHP-2008-0314-01-RV.

Gent DH, Nelson ME, Grove GG, Mahaffee WF, Turechek WW, Woods JL, 2012. Association of spring pruning practices with severity of powdery mildew and downy mildew on hop. *Plant Disease* **96,** 1343-51.

Grünwald NJ, Goss EM, 2011. Evolution and population genetics of exotic and re-emerging pathogens: novel tools and approaches. *Annual Review of Phytopathology* **49**, 249-67.

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95-8.

Homma Y, 1933. Homothallism in Sphaerotheca fuliginea (Schlecht.). *Proceedings of the Imperial Academy of Japan, Tokyo* **9**, 186-7.

Jarvis WR, Gubler WD, Grove GG, 2002. Epidemiology of powdery mildews in

agricultural pathosystems. In: Bélanger R, Bushnell WR, Did AJ, and Carver TLW, eds. *The Powdery Mildews, A Comprehensive Treatise*. St. Paul, MN: The American Phytopathological Society, 169-99.

Johansen DA, 1940. *Plant Microtechnique*. New York: McGraw-Hill.

Liyanage A, Royle DJ, 1976. Overwintering of *Sphaerotheca humuli*, the cause of hop powdery mildew. *Annual of Applied Biology* **83**, 381-94.

McDonald DA, Linde C, 2002. Pathogen population and genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology* **40**, 349-79.

Neve RA, 1991. *Hops*. London, UK: Chapman and Hall.

Ocamb CM, Klein R, Barbour J, Griesbach J, Mahaffee W, 1999. First report of hop powdery mildew in the Pacific Northwest. *Plant Disease* **83**, 1072.

Royle DJ, 1976. Plant Pathology Section, the years work, In: Wye College Department of Hop Research and Annual Report. April 1976 to December 1976. pp 22-5

Royle DJ, 1978. Powdery mildew of the hop. In: Spencer DM, ed. *The Powdery Mildews*. London, UK: Academic Press, 381-409.

Smith CG, 1970. Production of powdery mildew cleistocarps in a controlled environment. *Transactions of the British Mycological Society* **55**, 355-365.

Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stber K, van Themaat EVL, Brown JKM, Butcher SA, Gurr SJ, Lebrun MH, Ridout CJ, Schulze-Lefert P, Talbot NJ, Ahmadinejad N, Ametz C, Barton GR, Benjdia M, Bidzinski P, Bindschedler LV, Both M, Brewer MT, Cadle-Davidson L, Cadle-Davidson MM, Collemare J, Cramer R, Frenkel O, Godfrey D, Harriman J, Hoede C, King BC, Klages S, Kleemann J, Knoll D, Koti PS, Kreplak J, López-Ruiz FJ, Lu X, Maekawa T, Mahanil S, Micali C, Milgroom MG, Montana G, Noir S, O'Connell RJ, Oberhaensli S, Parlange F, Pedersen C, Quesneville H, Reinhardt R, Rott M, Sacristán S, Schmidt SM, Schön M, Skamnioti P, Sommer H, Stephens A, Takahara H, Thordal-Christensen H, Vigouroux M, Weßling R, Wicker T, Panstruga R, 2010. Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science* **330**, 1543–46.

Tollenaere C, Laine AL, 2013. Investigating the production of sexual resting structures in a plant pathogen reveals unexpected self-fertility and genotype-by-environment effects. *Journal of Evolutionary Biology* doi: 10.1111/jeb.12169.

Turgeon BG, Yoder OC, 2000. Proposed nomenclature for mating type genes of filamentous Ascomycetes. *Fungal Genetics and Biology* **31**,1-5.

White TJ, Bruns T, Lee S, TaylorJ, 1990. Amplificationanddirect sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols. A Guide to Methods and Applications. San Diego, CA, USA: Academic Press, 29–50.

Wicker T, Oberhaensli S, Parlange F, Buchmann JP, Shatalina M, Roffler S, Ben-David R, Doležel J, Šimková H, Schulze-Lefert P, Spanu PD, Bruggmann R, Amselem J, Quesneville H, Ver Loren van Themaat E, Paape T, Shimizu KK, Keller B, 2013. The wheat powdery mildew genome shows the unique evolution of an obligate biotroph. *Nature Genetics* **45**, 1092–6

Wolfenbarger SN, Eck EB, Ocamb CM, Probst C, Nelson ME, Grove GG, Gent DH, 2013. Powdery mildew outbreaks caused by Podosphaera macularis on hop cultivars possessing the resistance gene R6 in the Pacific Northwestern US. *Plant Disease* doi:10.1094/PDIS-11-13-1127-PDN.

Zerbino DR, Birney E, 2008. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. *Genome Research* **18**, 821–2.

Table 1. Presence and absence of cleistothecia in different pairings of *Podosphaera macularis* isolates putatively identified by PCR assays for *MAT1* locus

Powdery mildew Isolate	PNW (*MAT1-1*)	Tester 1 (*MAT1-1*)	Tester 2 (*MAT1-2*)
PNW (*MAT1-1*)	-	-	+
Tester 1 (*MAT1-1*)	-	-	+
Tester 2 (*MAT1-2*)	+	+	-

Table 2. Survey for *MAT1* locus within *Podosphaera macularis* samples from the Pacific Northwest[a]

	State	Total number of isolates	Number of locations[b]	*MAT1-1*	*MAT1-2*
2012	OR	68	10	68	0
	WA	85	19	85	0
	ID	30	6	30	0
2013	OR	60	12	60	0
	WA	74	14	74	0
	Total	317	56	317	0

[a] Samples were collected in early, mid, and late season and maintained for 3 to 4 weeks on a powdery mildew susceptible cultivar. All isolates were screened for the presence or absence of *MAT1-1* and *MAT1-2* by PCR. The resulting ratio for *MAT1-1* to *MAT1-2* was 1:0 (Chi square test for 1:1 ratio rejected at $P < 0.0001$).

[b] The total number of locations is the number of unique locations sampled. Some sites were sampled multiple times over the two years of this study.

Table 3. Survey for mating type locus in *Podosphaera macularis* samples from sexually reproducing populations

Location[a]	Number of samples	Number of samples with cleistothecia at time of sampling	*MAT1-1*	*MAT1-2*
Maryland	14	14	14	14
New York	17	8	11	14
Minnesota	4	0	1	3
Germany	4	0	0	4
Germany *	5	0	3	2
England*	2	0	1	1
France*	1	0	1	0
Slovenia	5	1	4	2
Czech Republic	4	NA	3	4
Total	**56**	**23**	**38**	**44**

[a] Locations with an asterisk (*) indicate clonal (single conidial chain) isolates were

assayed, whereas the others are from heterogeneous inoculum sources. The resulting

ratio for *MAT1-1* to *MAT1-2* was 1:1 (Chi square test *P* = 0.508). Samples from the

Czech Republic were received as DNA.

Table 4. Formation of cleistothecia by sexually compatible isolates of *Podosphaera macularis* in physical pairings at different temperature regimes

Temperature Regime	High (°C)	Low (°C)	Lesions with cleistothecia (%)[a]
Pacific Northwest climate	27.9	11	38.4a
Constant 13°C	13	13	44.1ab
Room temperature	26	16	52.7b

[a] Pairings of clonal Pacific Northwest isolates with tester *MAT1-2* grown in different

temperature regimes. Means with same letters are not significantly different

according to a generalized linear mixed model analysis (temperature treatment *F* =

3.81; *P* = 0.0395)
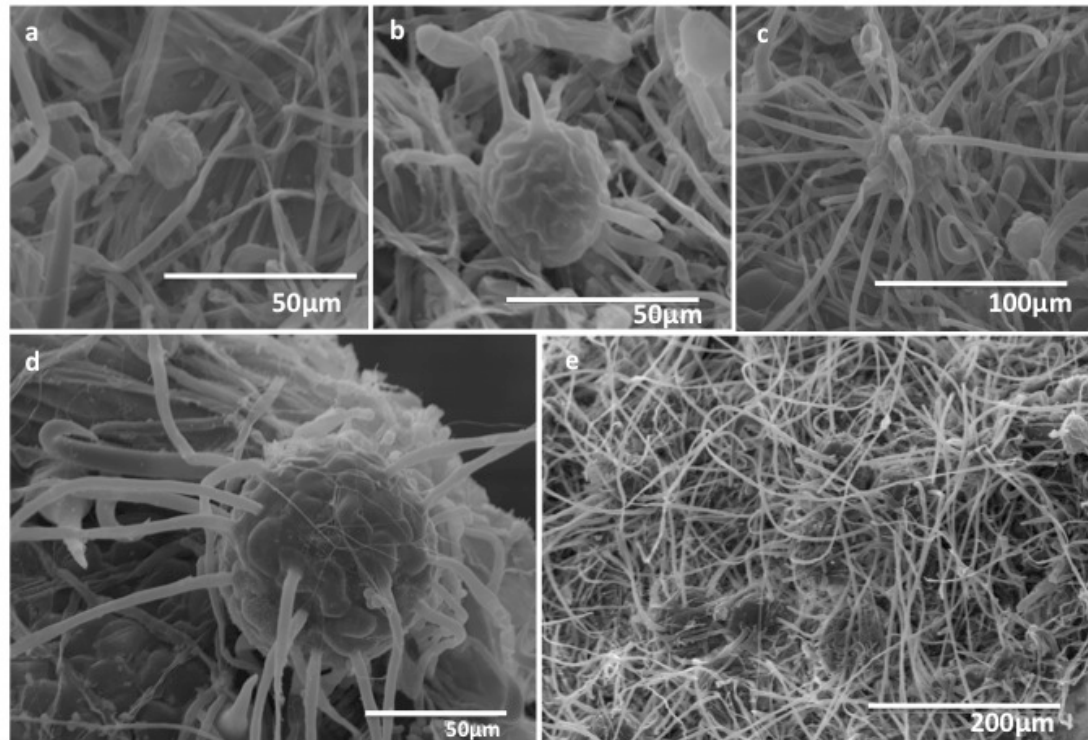
Figure 1. Development of cleistothecia of *Podosphaera macularis* documented with scanning electron microscopy. (a) An initial about 15µm in diameter; (b) Cleistothecium approximately 25 µm in diameter beginning to form appendages; (c) Cleistothecium approximately 50 µm in diameter; (d) Mature cleistothecia approximately 90µm in diameter; (e) Lesion with numerous cleistothecia with appendages embedded in and intertwined with pannose mycelia anchoring the ascocarps to the affected leaf.

Figure 2. Ascospore viability of *Podosphaera macularis* (photos provided by David Gadoury). (a) crush mount of cleistothecia; (b) crush mount of cleistothecia in "a" stained with FDA demonstrating viability of asci and ascospores; (c) crush mount of cleistothecium; (d) crush mount of cleistothecium in "c" stained with Sudan Black B showing staining of lipids; (e) 2 week old colony derived from ascospore infection.

GENERAL CONCLUSIONS

Podosphaera macularis continues to be one of the most economically important pathogens of hop. Having a better understanding of the basic overwintering biology is crucial for creating successful management strategies. The main objective of this project was to identify why the teleomorph of *P. macularis* is not observed in the Pacific Northwest while being present in other populations throughout North America and Europe. *P. macularis* was demonstrated to be heterothallic through PCR assay and physical pairings. A survey of *P. macularis* samples from the Pacific Northwest detected only the *MAT1-1* idiomorph in the population, providing evidence that the lack of the ascigerious stage can be explained by the lack of the *MAT1-2* idiomorph in the population. Strains of the pathogen originating from the Pacific Northwest were capable of sexual reproduction with isolates from outside the region and temperatures typical of late summer when hop cones are maturing appear permissive for ascocarp initiation. Examination of the structure of the *MAT1* locus in *P. macularis* of a *MAT1-1* isolate, along with ascocarp maturation and FDA staining, revealed similarities to other powdery mildew fungi know to produce viable ascospores. Ascospores formed by *P. macularis* were eventually demonstrated to be infective after overwintering. With information provided by this research quarantine regulations in Oregon, Washington and Idaho have been updated to prohibit the importation of hop to the region in an attempt to prevent the introduction of strains of *P. macularis* with the second mating type (*MAT1-2)* to the region.

BIBLIOGRAPHY

Abouelhoda M, Kurtz S, Ohlebusch E, 2004. Replacing suffix trees with enhanced suffix arrays. *Journal of Discrete Algorithms* **2**, 53–86.

Agrios, GN, 2005. *Plant Pathology*, 5th *edition*. San Diego, California: Elsevier-Academic Press.

Aslaf B, Gadoury DM, Tronsmo AM, Seem RC, Cadle-Davidson L, Brewer MT, Stensvand A, 2013. Temperature regulates the initiation of chasmothecia in powdery mildew of strawberry. *Phytopathology* **103**, 717-24.

Bardin M, Suliman ME, Sage-Palloix AM, Mohamed YF, Nicot PC, 2007. Inoculum production and long-term conservation methods for cucurbits and tomato powdery mildews. *Mycological Research* **111**, 740-47.

Barth HJ, Klinke C, Schmidt C, 1994. *The Hop Atlas*. Nuremberg, Germany: Joh Barth. and Sohn.

Bender CL, Coyier DL,1985. Heterothallism in *Sphaerotheca pannosa* var. *rosae*. *Transactions of the British Mycological Society* **84**, 647-52.

Blogett FM, 1913. Hop Mildew. Bulletin Cornell University Agricultural Experiment Station **328**, 278- 310.

Braun U, 1987. A monograph of the Erysiphales (powdery mildews). Beih.Nova Hedw. **89** 1–700

Braun U, Cook TA, Inman AJ, Shin HD, 2002. The taxonomy of the powdery mildew fungi. In: Bélanger R, Bushnell WR, Did AJ, and Carver TLW, eds. *The Powdery Mildews, A Comprehensive Treatise*. St. Paul, MN: The American Phytopathological Society, 1-12.

Brewer MT, Milgroom MG, 2010. Phylogeography and population structure of the grape powdery mildew fungus, *Erysiphe necator,* from diverse *Vitis* species. *BMC Evolutionary Biology* **10**, 268.

Brewer MT, Cadle-Davidson L, Cortesi P, Spanu PD, Milgroom MG, 2011. Identification and structure of the mating-type locus and development of PCR-based markers for mating type in powdery mildew fungi. *Fungal Genetics Biology* **48**, 704-13.

Brown JKM, Hovmoller MS, 2002. Aerial dispersal of pathogens on the global and continental scales and its impact on plant disease. *Science* **297**, 537- 41.

Burdon JJ, Roelfs AP, 1985. The effect of sexual and asexual reproduction on the isozyme structure of populations of Puccinia graminis. *Phytopathology* **75**, 1068-73.

Burgess A, 1964. *Hops: Botany, Cultivation, and Utilization.* Interscience Publishers, New York.

Bushnell WR, 2002. The role of powdery mildew research in understanding host-parasite interaction: past, present, and future. In: Bélanger R, Bushnell WR, Did AJ, and Carver TLW, eds. *The Powdery Mildews, A Comprehensive Treatise*. St. Paul, MN: The American Phytopathological Society, 1-12.

Butt DJ, 1972. The timing of sprays for the protection of terminal buds on apple shoots from powdery mildew. *Annual Applied Biology* **72**, 239-48.

Caffi T, Legler SE, Bugiani R, Rossi V, 2013. Combining sanitation and disease modelling for control of grapevine powdery mildew. *European Journal of Plant Pathology* **135**, 817-29.

Calonnec A, Cartolaro P, Poupot C, Dubourdieu D, Darriet P, 2004. Effects of *Uncinula necator* on the yield and quality of grapes (*Vitis vinifera*) and wine. *Plant Pathology* **53,** 434-45.

Celio GJ, Hausbeck MK, 1998. Conidial germination, infection structure formation, and early colony development of powdery mildew on poinsettia. *Phytopathology* **88**, 105-13.

Cherewick WJ, 1944. Studies on the biology of *Erysiphe graminis* DC. *Canadian Journal of Research* **22**, 25-86.

Clifford DR, Gendle P, Holgate ME, Lulham M, 1981. Eradication of over-wintering mildew (*Podosphaera leucotricha*) in apple buds by nitrogen-containing surfactants. *Pesticide Science* **12**, 509-14.

Coley-Smith DJ, 1964. An association between so-called premature ripening of hops and the presence of powdery mildew (*Sphaerotheca macularis*). *Department of Hop Research Annual Report-Wye College* **1963,** 30-1.

Coyier DL, 1973. Heterothallism in the apple powdery mildew fungus, *Podosphaera leucotricha*. *Phytopathology* **64**, 246-48.

Darby P. 2013. Could there be a dominant gene for susceptibility to hop powdery mildew?. *International Hop Growers Convention*,17-20.

Dean R, Van Kan JAL, Pretorius ZA, Hammond-Kosack KE, Di Pietro A, Spanu P D, Rudd JJ, Dickman M, Kahmann R, Ellis J, Foster GD, 2012. The top 10 fungal pathogens in molecular plant pathology. *Molecular Plant Pathology* **13**, 414-30.

Debuchy R,Turgeon BG, 2006. Mating-type structure, evolution, and function in Euascomycetes, In: Kües U, Fisher R eds. *The Mycota vol I. Growth, Differentiation and Sexuality*, Berlin, Germany: Springer Berlin Heidelberg, 293-323.

De Wit CT, Goudriaan J, van Laar HH, Penning de Vries FWT, Rabbinge R, van Keulen H, Sibma L, de Jonge C, 1978. Simulation of assimilation, respiration and transpiration of crops. Pudoc, Wageningen, The Netherlands: Simulation Monographs. pp 141.

Feldbrügge M, Kämper J, Steinberg G, Kahmann R, 2004. Regulation of mating and pathogenic development in *Ustilago maydis*. *Current Opinion Microbiology* **7**, 666–72.

Füzi, I. 2003. Natural parasitism of *Uncinula necator* cleistothecia by *Ampelomyces* hyperparasites in the south-western vineyards of Hungary. *Acta phytopathologica et entomologica hungarica* **38**, 53-60.

Gadoury DM, Pearson RC, 1988. Initiation, development, dispersal, and survival of cleistothecia *Uncinula necator* in New York vineyards. *Phytopathology* **78**, 1413-21.

Gadoury DM, Pearson RC, 1990. Ascocarp dehiscence and ascospore discharge in *Uncinula necator*. *Phytopathology* **80**, 393-401.

Gadoury DM, Pearson RC, 1991. Heterothallism and pathogenic specialization in *Uncinula necator*. *Phytopathology* **81**, 1287-93.

Gadoury DM, Seem RC, Ficke A, Wilcox WF, 2003. Ontogenic resistance to powdery mildew in grape berries. *Phytopathology* **93**, 547-55.

Gadoury DM, Asalf B, Heidenreich MC, Herrero ML, Welser MJ, Seem RC, Tronsmo AM, Stensvand A, 2010. Initiation, development, and survival of cleistothecia of *Podosphaera aphanis* and their role in the epidemiology of strawberry powdery mildew. *Phytopathology* **100**, 246-51.

Gadoury DM, Cadle-Davidson L, Wilcox WF, Dry IB, Seem RC, Milgroom MG, 2011. Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for

the study of the biology, ecology and epidemiology of an obligate biotroph. *Molecular Plant Pathology* **13**, 1-16.

Gaunt RE, 1995. The relationship between plant disease severity and yield. *Annual Review of Phytopathology* **33**, 119-44.

Gent DH, Mahaffee WF, Turechek WW, 2006. Spatial heterogeneity of the incidence of powdery mildew on hop cones. *Plant Disease* **90**, 1433-40.

Gent DH, Nelson ME, George AE, Grove GG, Mahaffee WF, Ocamb CM, Barbour JD, Peetz A, Turechek WW. 2008. A decade of hop powdery mildew in the pacific northwest. Online. *Plant Health Progress* doi:10.1094/PHP-2008-0314-01-RV.

Gent DH, Nelson ME, Grove GG, Mahaffee WF, Turechek WW, Woods JL, 2012. Association of spring pruning practices with severity of powdery mildew and downy mildew on hop. *Plant Disease* **96,** 1343-51.

Gent DH, Grove GG, Nelson ME, Wolfenbarger SN, Woods JL, 2013. Crop damage caused by powdery mildew on hop and its relationship to late season management. *Plant Pathology* doi: 10.1111/ppa.12123.

Glawe DA, 2003. First report of powdery mildew of *Nandina domestica* caused by *Microsphaera berberidis* (*Erysiphe berberidis*) in the Pacific Northwest. Online. *Plant Health Progress* doi:10.1094/PHP-2003-1023-01-HN.

Glawe DA, 2008. The powdery mildews: a review of the world's most familiar (yet poorly known) plant pathogens. *Annual Review of Phytopathology* **46**, 27-51.

Gordon CC, 1966. A reinterpretation of the ontogeny of the ascocarp of species of the Erysiphaceae. *American Journal of Botany* **53**, 652-62.

Green JR, Carver TLW, Gurr SJ, 2002. The formation and function of infection and feeding structures. In: Bélanger R, Bushnell WR, Did AJ, and Carver TLW, eds. *The Powdery Mildews, A Comprehensive Treatise*. St. Paul, MN: The American Phytopathological Society, 66-82.

Grünwald NJ, Goss EM, 2011. Evolution and population genetics of exotic and re-emerging pathogens: novel tools and approaches. *Annual Review of Phytopathology* **49**, 249-67.

Hall TA, 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* **41**, 95-8.

Homma Y, 1933. Homothallism in Sphaerotheca fuliginea (Schlecht.). *Proceedings of the Imperial Academy of Japan, Tokyo* **9**, 186-87.

Homma Y, 1937. Erysiphaceae of Japan. *Journal of the Faculty of Agriculture, Hokkaido University* **38**, 183-461.

Hysert D, 2009. Cone use and chemistry. In: Mahaffee WF, Pethybridge SJ, Gent DH, eds. *Compendium of Hop Diseases and Pests*. St Paul, MN, USA: APS Press, 8–11.

Jarvis WR, Gubler WD, Grove GG, 2002. Epidemiology of powdery mildews in agricultural pathosystems. In: Bélanger R, Bushnell WR, Did AJ, and Carver TLW, eds. *The Powdery Mildews, A Comprehensive Treatise*. St. Paul, MN: The American Phytopathological Society, 169-99.

Johansen DA, 1940. *Plant Microtechnique*. New York: McGraw-Hill.

Kavalier AR, Litt A, Ma C, Pitra NJ, Coles MC, Kennelly EJ, Matthews PD, 2011. Phytochemical and morphological characterization of hop (*Humulus lupulus* L.) cones over five developmental stages using high performance liquid chromatography coupled to time-of-flight mass spectrometry, ultrahigh performance liquid chromatography photodiode array detection, and light microscopy techniques. *Journal of Agricultural and Food Chemistry* **59**, 4783–93.

Legler SE, Caffi T, Rossi V, 2013. A model for the development of *Erysiphe necator* chasmothecia in vineyards. Online. *Plant Pathology* doi: 10.1111/ppa.12145.

Li B, Cao X, Chen L, Zhou Y, Duan X, Luo Y, Fitt BDL, Xu X, Song Y, Wang B, Cao S, 2013. Application of geographic information systems to identify the oversummering regions of *Blumeria graminis* f. sp. *tritici* in China. *Plant Disease* **97**:1168-1174.

Lipps PE, Madden LV, 1989. Assessment of methods of determining powdery mildew severity in relation to grain yield of winter wheat cultivars in Ohio. *Phytopathology* **79**, 462-70.

Liyanage A, 1973. Studies on resistance and overwintering in hop powdery mildew (*Sphaerotheca humuli*). Ph.D. thesis. University of London.

Liyanage A, Royle DJ, 1976. Overwintering of *Sphaerotheca humuli*, the cause of hop powdery mildew. *Annual of Applied Biology* **83**, 381-94.

Luttrell ES, 1951. Taxonomy of Pyrenomycetes. *University of Missouri studies* **24**, 1-20.

Mahaffee WF, Turechek WW, Ocamb CM, 2003. Effect of variable temperature on infection severity of *Podosphaera macularis* on hops. *Phytopathology* **93,** 1587-92.

Mahaffee WF, Thomas CS, Turechek WW, Ocamb CM, Nelson ME, Fox A, Gubler WD, 2003. Responding to an introduced pathogen: *Podosphaera macularis* (hop powdery mildew) in the Pacific Northwest. Online. *Plant Health Progress* doi:10.1094/PHP-2003-1113-07-RV.

McDonald DA, Linde C, 2002. Pathogen population and genetics, evolutionary potential, and durable resistance. *Annual Review of Phytopathology* **40**, 349-79.

McGrath MT, 1994. Heterothallism in *Sphaerotheca fuliginea*. *Mycologia* **86**, 517-523.

Miazzi M, Laguardia C, Faretra F, 2011. Variation in *Podosphaera xanthii* on cucurbits in southern Italy. *Journal of Phytopathology* **159**, 538-45.

Montarry J, Cartolaro P, Delmotte F, Jolivet J, Willocquet L, 2008. Genetic structure and aggressiveness of *Erysiphe necator* populations during grapevine powdery mildew epidemics. *Applied and Environmental Microbiology* **74**, 6327-32.

Neve RA, 1991. *Hops*. London, UK: Chapman and Hall.

Ocamb CM, Klein R, Barbour J, Griesbach J, Mahaffee W, 1999. First report of hop powdery mildew in the Pacific Northwest. *Plant Disease* **83**, 1072.

Pearson RC, Gärtel, W, 1985. Occurrence of hyphae of *Uncinula necator* in buds of grapevine. *Plant Disease* **69**, 149-51.

Peetz AB, Mahaffee WF, Gent DH, 2009. Effect of temperature on sporulation and infectivity of *Podosphaera macularis* on *Humulus lupulus*. *Plant Disease* **93**, 281-86.

Perez-Garcia A, Romero D, Fernandez-Ortuno D*, et al.*, 2009. The powdery mildew fungus Podosphaera fusca (synonym *Podosphaera xanthii*), a constant threat to cucurbits. *Molecular Plant Pathology* **10**, 153-160.

Royle DJ, 1976. Plant Pathology Section, the years work, In: Wye College Department of Hop Research and Annual Report. April 1976 to December 1976. pp 22-25

Royle DJ, 1978. Powdery mildew of the hop. In: Spencer DM, ed. *The Powdery Mildews*. London, UK: Academic Press, 281-409.

Rybáček V, ed. 1991. *Hop Production*. Elsevier Science Publishing Company, New York.

Sall MA, Wrysinski, J, 1982. Perennation of powdery mildew in buds of grapevines. *Plant Disease* **66**, 678-79.

Seigner E, Seefelder S, Haugg B, Engelhard B, Hasyn S, Felsenstein FG, 2003. Potential of powdery mildew (*Sphaerotheca humuli*) to infect hops (*Humulus lupulus*) in various developmental stages. *Gesunde Pflanzen* 55, 29–33.

Smith CG, 1970. Production of powdery mildew cleistocarps in a controlled environment. *Transactions of the British Mycological Society* **55**, 355-365.

Spanu PD, Abbott JC, Amselem J, Burgis TA, Soanes DM, Stber K, van Themaat EVL, Brown JKM, Butcher SA, Gurr SJ, Lebrun MH, Ridout CJ, Schulze-Lefert P, Talbot NJ, Ahmadinejad N, Ametz C, Barton GR, Benjdia M, Bidzinski P, Bindschedler LV, Both M, Brewer MT, Cadle-Davidson L, Cadle-Davidson MM, Collemare J, Cramer R, Frenkel O, Godfrey D, Harriman J, Hoede C, King BC, Klages S, Kleemann J, Knoll D, Koti PS, Kreplak J, López-Ruiz FJ, Lu X, Maekawa T, Mahanil S, Micali C, Milgroom MG, Montana G, Noir S, O'Connell RJ, Oberhaensli S, Parlange F, Pedersen C, Quesneville H, Reinhardt R, Rott M, Sacristán S, Schmidt SM, Schön M, Skamnioti P, Sommer H, Stephens A, Takahara H, Thordal-Christensen H, Vigouroux M, Weßling R, Wicker T, Panstruga R, 2010. Genome expansion and gene loss in powdery mildew fungi reveal tradeoffs in extreme parasitism. *Science* **330**, 1543–46.

Spotts RA, Chen PM, 1984. Cold hardiness and temperature responses of healthy and mildew infected terminal buds of apple during dormancy. *Phytopathology* **74**, 542-44.

Takamatsu S, 2004. Phylogeny and evolution of the powdery mildew fungi (Erysiphales, Ascomycota) inferred from nuclear ribosomal DNA sequence. *Mycoscience* **45**, 147-57.

Takamatsu S, 2012. Origin and evolution of powdery mildews (Ascomycota, Erysiphales). *Mycoscience* **54**, 75-86

Tollenaere C, Laine AL, 2013. Investigating the production of sexual resting structures in a plant pathogen reveals unexpected self-fertility and genotype-by-environment effects. *Journal of Evolutionary Biology* doi: 10.1111/jeb.12169.

Turechek WW, Mahaffee WF, Ocamb CM, 2001. Development of management strategies for hop powdery mildew in the Pacific Northwest. Online. *Plant Health Progress doi:*10.1094/PHP-2001-0313-01-RS.

Turgeon, Yoder OC, 2000. Proposed nomenclature for mating type genes of filamentous Ascomycetes. *Fungal Genetics and Biology* **31**,1-5.

USDA. 2013. US hop statistics. USDA-National Agricultural Statistics Service. Online. http://www.usahops.org/userfiles/image/1388461395_National%20Hop%20Report-NASS%2012-13.pdf (verified 30 March 2014).

Welz G, Kranz J, 1987. Effects of recombination on races of a barley powdery mildew population. *Plant Pathology* **36**, 107-13.

White TJ, Bruns T, Lee S, TaylorJ, 1990. Amplificationanddirect sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols. A Guide to Methods and Applications. San Diego, CA, USA: Academic Press, 29–50.

Wicker T, Oberhaensli S, Parlange F, Buchmann JP, Shatalina M, Roffler S, Ben-David R, Doležel J, Šimková H, Schulze-Lefert P, Spanu PD, Bruggmann R, Amselem J, Quesneville H, Ver Loren van Themaat E, Paape T, Shimizu KK, Keller B, 2013. The wheat powdery mildew genome shows the unique evolution of an obligate biotroph. *Nature Genetics* **45**, 1092–96

Wolfe MS, 1984. Trying to understand and control powdery mildew. *Plant pathology* **33**, 451-66.

Wolfe MS, Schwarzbach E, 1978. Patterns of race changes in powdery mildews. *Annual Review of Phytopathology* **16**, 159-80.

Wolfenbarger SN, Nelson ME, Grove GG, Woods JL, Gent DH, 2012. Characterization of ontogenic resistance to powdery mildew in hop cones. *Phytopathology* **102,** S4.137.

Wolfenbarger SN, Eck EB, Ocamb CM, Probst C, Nelson ME, Grove GG, Gent DH, 2013. Powdery mildew outbreaks caused by *Podosphaera macularis* on hop cultivars possessing the resistance gene R6 in the Pacific Northwestern US. *Plant Disease* doi:10.1094/PDIS-11-13-1127-PDN.

Woods JL, Nelson ME, Grove GG, Gent DH, 2010. Ontogenic resistance to powdery mildew in hop cones: Implications for disease management. *Phytopathology* S.138

Yarwood CE, 1957. Powdery mildews. *Botany Review* **33**, 235-301.

Ypema HL, Gubler WD, 2000. The distribution of early season grapevine shoots infected by *Uncinula necator* from year to year: a case study in two California vineyards. *American Journal of Enology and Viticulture* **51**, 1–6.

Zerbino DR, Birney E, 2008. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. *Genome Research* **18**, 821–2.

Zheng Q, Hou R, Juanyu, Zhang, Ma J, Wu Z, Wang G, Wang C, Xu JR, 2013. The *MAT* locus genes play different roles in sexual reproduction and pathogenesis in *Fusarium graminearum*. *PLoS ONE* **8** doi:10.1371/journal.pone.0066980

APPENDIX

Protocol for maintenance of *Podosphaera macularis* isolates

Materials

Biocontainment hood

Petri dishes (100mm x 15mm)

Made double Petri dishes

Water

70% ethanol in spray bottle

95% ethanol

Rooting solution

Susceptible plant (cv. Symphony)

Scissors

Dissecting microscope

Transfer tools

10% bleach water

Procedure

1. Select susceptible plants (cv. Symphony) grown in a powdery mildew free greenhouse that have young, unfurled leaves at the first node. Avoid leaves that are chlorotic or have damage (such as thrips damage). Surface of the leaves are misted with 70% ethanol and rinsed with water after 30 to 60 seconds for approximately 30 seconds to ensure potential sulfur and ethanol residues are removed. Allow plants to dry.

2. After plants are dry, detach the first node of the plant by cutting the stem approximately 1-2 inches below the leaves, also remove stem above the leaves. Dip the stem in rooting solution for 5 to 10 seconds and then place in a double Petri plate with water in bottom.

3. Infected tissue (generally leaves or cones) is brought in from the field. Infected areas of tissue are dabbed onto powdery mildew free leaves prepared above. Generally 3 to 4 leaves in individual double Petri plates are used per isolate. Newly inoculated cultures are placed in a growth chamber for 2 weeks to establish and sporulate. Alternatively, an isolate may already be growing and can be transferred as below.

4. Isolates should be transferred to new leaves every 2 to 3 weeks to ensure the health of the isolate. Isolates should be transferred in the biocontainment hood to decrease the probability of isolates becoming contaminated and to prevent escape of isolates from outside of Oregon as per APHIS permit requirements.

5. Under a dissecting scope transfer small quantities (1 to 10 chains) of *P. macularis* using either a small insect needle or eyelash transfer tool to a prepared leaf. Repeat 7 to 10 times per leaf. Transfer tools should be sterilized in 95% ethanol for at least 60 seconds between isolates. In addition hands and surfaces of hood should be sprayed with 70% ethanol between isolates.

6. Petri plate containing the newly inoculated leaf should be labeled and sealed with parafilm and placed in growth chamber.

7. Leaf containing the original culture can either be resealed and kept as a backup culture or discarded. If discarding the leaf should be removed from the double Petri plate and sealed in a plastic bag or in single Petri plate. The double Petri plate and water should be placed in 10% bleach water before being washed. The discarded leaves should be autoclaved before being thrown away.

Protocol for obtaining a clonal (single-chained) isolate of *Podosphaera macularis*

Materials

Infected plant material with sporulating colonies

Biocontainment hood

Dissecting microscope

Transfer tools

70% ethanol in spray bottle

95% ethanol

10% bleach water

Healthy leaves prepared in detach system (see maintenance of *P. macularis* isolates)

Procedure

1. Obtain an infected hop leaf with sporulating colonies of *P. macularis.* Pick a single colony that is healthy (no or few contaminates present). Sterilize biocontainment hood by spraying surface with 70% ethanol. Also sterilize transfer tools by soaking in 95% ethanol for at least 60 seconds.

2. In the biocontainment hood with a dissecting microscope use the transfer tool to select a single conidiophore from the previously selected colony. Conidiophores are easiest to isolate around the edge of the colonies. Move the conidiophore to the healthy leaf. Repeat 7 to 10 times. Transfer tools should be sterilized in 95% ethanol

for at least 60 seconds between isolates. In addition hands and surfaces of hood should be sprayed with 70% ethanol between isolates.

3. Petri dish containing the newly inoculated leaf should be labeled and sealed with parafilm and placed in growth chamber. Already infected hop leaf can either be resealed and placed back in the growth chamber as backup culture or can be discarded. If discarded the leaf should be removed from the double Petri dish and sealed in a plastic bag or in single Petri dish. The double Petri dish and water should be placed in 10% bleach water before being washed. The discarded leaves should be autoclaved before being thrown away.

4. In 10 to 14 days transfer conidia from only one of the colonies formed from the above inoculations to healthy hop leaves. These leaves should be labeled and sealed with parafilm and placed in growth chamber. The resulting infection will be a clonal isolate of *P. macularis* and can be maintained as described in "Protocol for maintenance of *Podosphaera macularis* isolates".

Protocol for chelex extraction of *Podosphaera macularis*

Adapted from:
 Brewer MT, Milgroom MG, 2010. Phylogeography and population structure of the
    grape powdery mildew fungus, *Erysiphe necator,* from diverse *Vitis* species.
    BMC Evolutionary Biology **10**, 268.

Materials

Biocontainment hood

Clear office tape

70% ethanol

Forceps

Sterile, 1.5mL microcentifuge tube

5% chelex (Sigma C7901-25G) in a sterile 50 mL Falcon-type tube

Sterile silica beads (400 micron)

P200 pipette and P200 filter tips

Vortex

Microcentrifuge

Block heater at 95°C

Negative 80 freezer

Procedure

1. Extraction works best with colonies 1 to 3 weeks old and free of contamination.

2. Working with gloves in a sterile biocontainment hood remove any exposed sticky part of office tape and wipe down tape dispenser with 70% ethanol. Using forceps pull about 2.5cm piece of tape and remove from dispenser.

3. Touch the tape to the mildew colony repeatedly until the tape is covered in conidia and no longer sticky.

4. Put the conidia-covered tape into a microcentrifuge tube and close the tube. Label tube with appropriate information.

5. Hood and gloves should be wiped down with 70% ethanol between each isolate being harvested for conidia. The above steps should be repeated with all isolates to be harvested for conidia. Once all samples are collected place tubes with tape at   -80°C overnight.

6. The following day tubes should be removed from the -80°C and work with in a hood to reduce contamination. Gloves should be worn when handling samples. Place 150ul of 5% chelex solution in each tube. Invert the chelex suspension several times to re-suspend chelex beads and quickly remove 150ul with a P200 filter tip with 1/3 of the tip removed with sterile scissors (to allow chelex beads to be drawn into pipette tip). Invert chelex between each tube to keep beads suspended.

7. Add about 10mg of silica beads to each tube.

8. Vortex tubes about 30 seconds, push tape down with sterile pipette tip if needed, vortex again for 30 seconds

9. Place tubes in a heat block set to 95°C for 15 minutes. Vortex tubes for 20 seconds and return them to the heat block for additional 15 minutes. Remove tubes from the heat block and spin in centrifuge for approximately 5 seconds (quick spin).

10. Let tubes cool at room temperature for approximately 30 minutes. This makes it easier to remove the supernatant but can be skipped if needed.

11. Remove supernatant from the tape and chelex beads with an uncut filter tip (usually about 50 to 100ul of liquid) and transfer to a new, sterile microcentrifuge tube. Label tube appropriately and store in -20°C. Use 1ul of solution in PCR reactions.

Protocol for softwood cutting of hop

Materials

Plants of desired hop cultivar

Scissors

Rooting hormone (see "rooting hormone solution" protocol)

Oasis tray wedge media

Solid-bottom tray

Water

Procedure

1. Place Oasis tray in a solid-bottom tray and fill about ½ full of water making sure the Oasis wedges are saturated.

2. Select plant material and remove bine from plant

3. Cut a single node (including leaves) from the removed bine. Remove one of the leaves and dip the stem end of the cutting in rooting hormone (do not get rooting hormone on the leaf). Insert cutting in Oasis wedge.

    Note: in hot weather it may be necessary to remove part of the remaining leaf to reduce transpiration and wilting of the cutting.

4. Label the tray with cultivar and date and make certain the tray is about ½ full of water on a regular basis.

5. Cuttings should be rooted and ready for up potting in about two to three weeks. When they are rooted, pot into desired size pot containing a slow release fertilizer. It is very important to cover the wedge with soil to prevent drying out, but it is also important to make sure the buds don't get covered up or else the cutting won't take.

Protocol for rooting hormone solution

Materials

2x 500mL glass bottles

latex or nitrile gloves

indole butyric acid

boric acid (granular)

scale

weigh boats

500 mL graduated cylinder

95% ethanol

deionized water

50mL beaker

Procedure

Before making these solutions, put on latex or nitrile gloves.

1. Indole butyric acid:

      a. add 1 gram of indole butyric acid to 500 mLs of 95% ethanol in a glass

bottle. Mix until dissolved.

2. Boric acid:

      a. add 1 gram of granular boric acid to 500mLs of deionized water in a glass

bottle. Mix until dissolved.

Store both of these solutions at 4°C

The same day softwood cuttings are being made:

1. Mix the solutions above at a 1:1 ratio for rooting hormone solution. In general, 10

mLs of each solution is mixed together in a small 50mL beaker.

Table 5. Samples of *Podosphaera macularis* from the Pacific Northwest

Number of samples	Date collected	State	City	Variety	Mating type
14	May 2011	OR	Corvallis	Breeding population	*MAT1-1*
5	September 2011	WA	Toppenish	Zeus	*MAT1-1*
4	March 2012	OR	Woodburn	Breeding population	*MAT1-1*
1	March 2012	WA	Yakima	Newport	*MAT1-1*
1	March 2012	WA	Yakima	Apollo	*MAT1-1*
1	March 2012	WA	Yakima	Nugget	*MAT1-1*
1	March 2012	WA	Yakima	Serebrianka	*MAT1-1*
5	May 2012	WA	Harrah	Bravo	*MAT1-1*
5	May 2012	WA	Prosser	Galena	*MAT1-1*
5	May 2012	WA	Prosser	Apollo	*MAT1-1*
5	May 2012	OR	Mt. Angel	Magnum	*MAT1-1*
5	May 2012	OR	Corvallis	Breeding population	*MAT1-1*
5	June 2012	OR	Silverton	Sterling	*MAT1-1*
5	June 2012	WA	Moxee	Cascade	*MAT1-1*
5	July 2012	WA	Moxee	CTZ	*MAT1-1*
5	July 2012	WA	Toppenish	Chelan	*MAT1-1*
4	July 2012	WA	Toppenish	Cluster	*MAT1-1*
4	July 2012	WA	Moxee	Nugget	*MAT1-1*
5	July 2012	OR	Hubbard	Willamette	*MAT1-1*
5	July 2012	OR	Corvallis	Symphony	*MAT1-1*
5	July 2012	WA	Moxee	CTZ	*MAT1-1*
5	July 2012	WA	Toppenish	Cascade	*MAT1-1*
12	September 2012	WA	Moxee	Apollo	*MAT1-1*
1	September 2012	WA	Mabton	Bravo	*MAT1-1*
5	September 2012	OR	Woodburn	Unknown	*MAT1-1*
5	September 2012	OR	Silverton	Sterling	*MAT1-1*
5	September 2012	OR	Mt. Angel	Magnum	*MAT1-1*
5	September 2012	OR	Silverton	Meridian	*MAT1-1*
20	September 2012	ID	Parma	Zeus	*MAT1-1*
10	September 2012	ID	Parma	Apollo	*MAT1-1*
5	September 2012	OR	Aurora	Breeding line	*MAT1-1*
10	September 2012	WA	Moxee	Chinook	*MAT1-1*
5	September 2012	WA	Moxee	Centennial	*MAT1-1*
5	April 2013	WA	Toppenish	Breeding line	*MAT1-1*
5	April 2013	WA	Prosser	Glacier	*MAT1-1*
5	May 2013	OR	Mt. Angel	Magnum	*MAT1-1*

| 5	May 2013	WA	Prosser	Apollo	*MAT1-1*
5	May 2013	WA	Toppenish	Summit	*MAT1-1*
5	May 2013	WA	Harrah	Bravo	*MAT1-1*
5	May 2013	OR	Mt. Angel	Santiam	*MAT1-1*
5	May 2013	OR	St. Paul	Unknown	*MAT1-1*
5	May 2013	WA	Toppenish	Cascade	*MAT1-1*
5	June 2013	OR	Mt. Angel	Super Galena	*MAT1-1*
5	June 2013	OR	Hubbard	Tettnanger	*MAT1-1*
5	June 2013	OR	Silverton	Willamette	*MAT1-1*
5	June 2013	OR	Hubbard	Willamette	*MAT1-1*
10	June 2013	WA	Toppenish	Cascade	*MAT1-1*
10	June 2013	WA	Moxee	CTZ	*MAT1-1*
4	June 2013	WA	Toppenish	CTZ	*MAT1-1*
5	July 2013	OR	Silverton	Cascade	*MAT1-1*
5	July 2013	OR	St. Paul	Centennial	*MAT1-1*
5	August 2013	WA	Toppenish	Centennial	*MAT1-1*
10	August 2013	OR	Corvallis	Symphony	*MAT1-1*
5	August 2013	OR	Woodburn	Nugget	*MAT1-1*
10	August 2013	WA	Toppenish	CTZ	*MAT1-1*
5	August 2013	WA	Toppenish	El Dorado	*MAT1-1*

[a] At least 5 infected tissues (leaves or cones) of hop were collected in the field and brought back to the laboratory for processing. Samples were transferred to susceptible detached leaves and grown for at least 2 weeks before conidia were collected for DNA extraction.

Table 6: Samples of *Podosphaera macularis* from outside the Pacific Northwest

Number of samples	Date collected	Location collected	GPS coordinates	Original cultivar	Mating type
1	1999	Hallertau, Germany	.	.	*MAT1-2*
1	1999	Hallertau, Germany	.	.	*MAT1-1*
1	1999	Hallertau, Germany	.	.	*MAT1-1*
1	2008	Hallertau, Germany	.	.	*MAT1-2*
1	1999	Hallertau, Germany	.	.	*MAT1-1*
4	March 2013	Germany	.	.	*MAT1-2*
1	1999	France	.	.	*MAT1-1*
1	2000	England	.	.	*MAT1-2*
1	2004	England	.	.	*MAT1-1*
1	August 2012	Seneca Castle, NY	.	.	*MAT1-1*
3	August 2012	Seneca Castle, NY	.	.	*MAT1-2*
3	October 2012	Seneca Castle, NY	.	.	*MAT1-1*
2	October 2012	Seneca Castle, NY	.	.	*MAT1-2*
8	October 2012	Seneca Castle, NY	.	.	*MAT1-1/MAT1-2*
4	October 2012	Sharpsburg, MD	39.462969, -77.7754	Feral hop	*MAT1-1/MAT1-2*
3	October 2012	Sharpsburg, MD	39.433873, -77.796214	Feral hop	*MAT1-1/MAT1-2*
2	October 2012	Sharpsburg, MD	39.437121, -77.800548	Feral hop	*MAT1-1/MAT1-2*
1	October 2012	Sharpsburg, MD	39.4444, -77.783494	Feral hop	*MAT1-1/MAT1-2*
4	October 2012	Sharpsburg, MD	39.430127, -77.76407	Feral hop	*MAT1-1/MAT1-2*
1	July 2013	Waseca, MN	.	Breeding line	*MAT1-1*
3	July 2013	Waseca, MN	.	Breeding line	*MAT1-2*
1	2012	Zalec, Slovenia	.	Magnum	*MAT1-1/MAT1-2*
3	September 2013	Savinja valley Slovenia	.	Dana	*MAT1-1*
1	September 2013	Zalec, Slovenia	.	Celeia	*MAT1-2*
1	October 2011	Czech Republic	.	.	*MAT1-1*
3	October 2011	Czech Republic	.	.	*MAT1-1/MAT1-2*

[a] Samples were collected and sent to Corvallis, OR for processing. When possible, samples were kept alive by transferring to mildew free hop leaves of cultivar Symphony and spores were collected for DNA extraction. When this was a not possible, leaf disks were removed from the infected sample and DNA extracted using UltraClean GelSpin DNA Extraction Kit, Mo Bio. Periods indicates missing information for those samples.

Figure 3. Alignment of *APN2*. Amino acid alignment (performed by Geneious version 6.0.5 [Biomatters]) of *APN2* to demonstrate similarities between closely related species. Top sequence is *Podosphaera macularis*, followed by *Blumeria graminis* f. sp. *hordei DH14, Botrytonia fuckeliana BcDW1,* and *Sclerotinia borealis F-4157*.
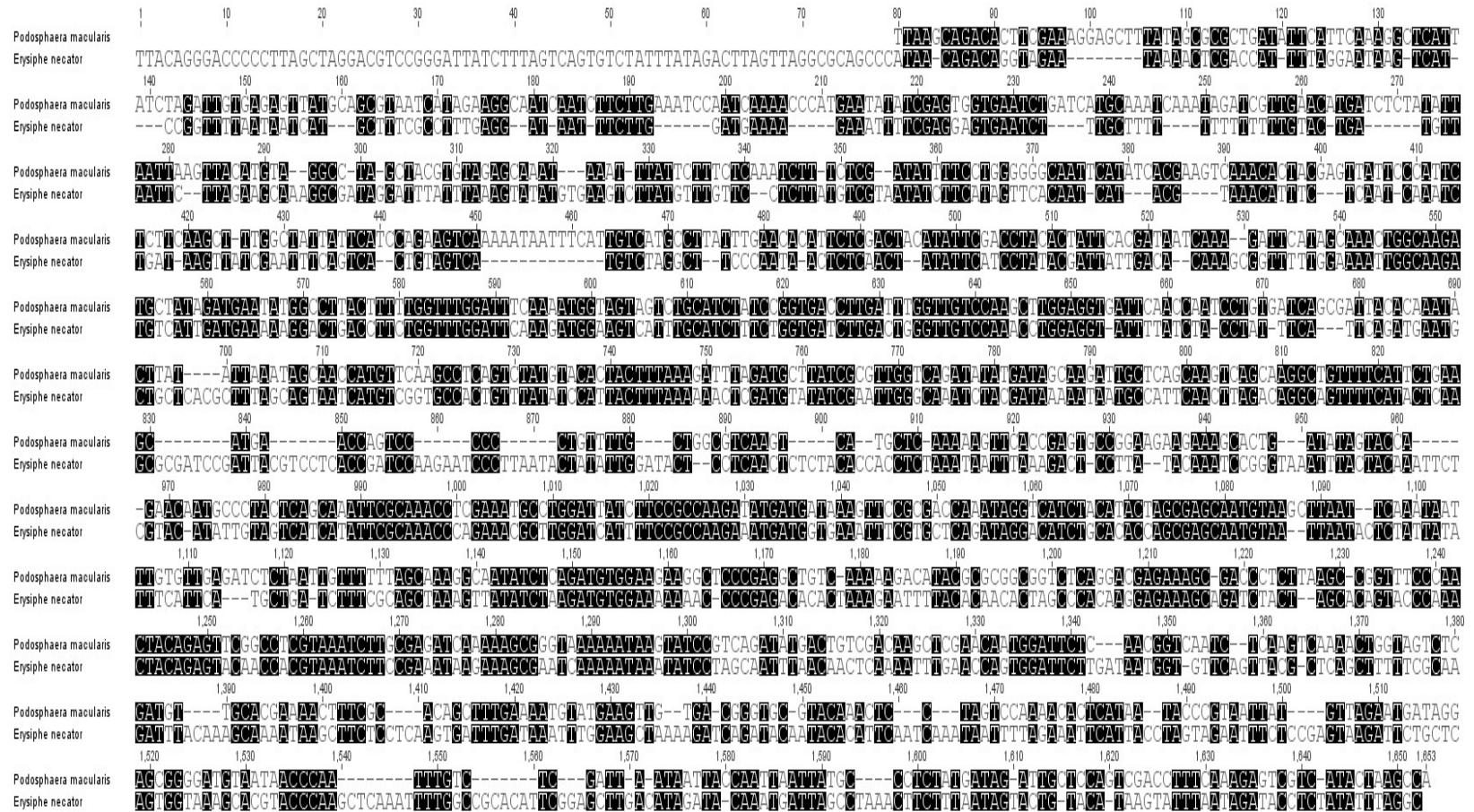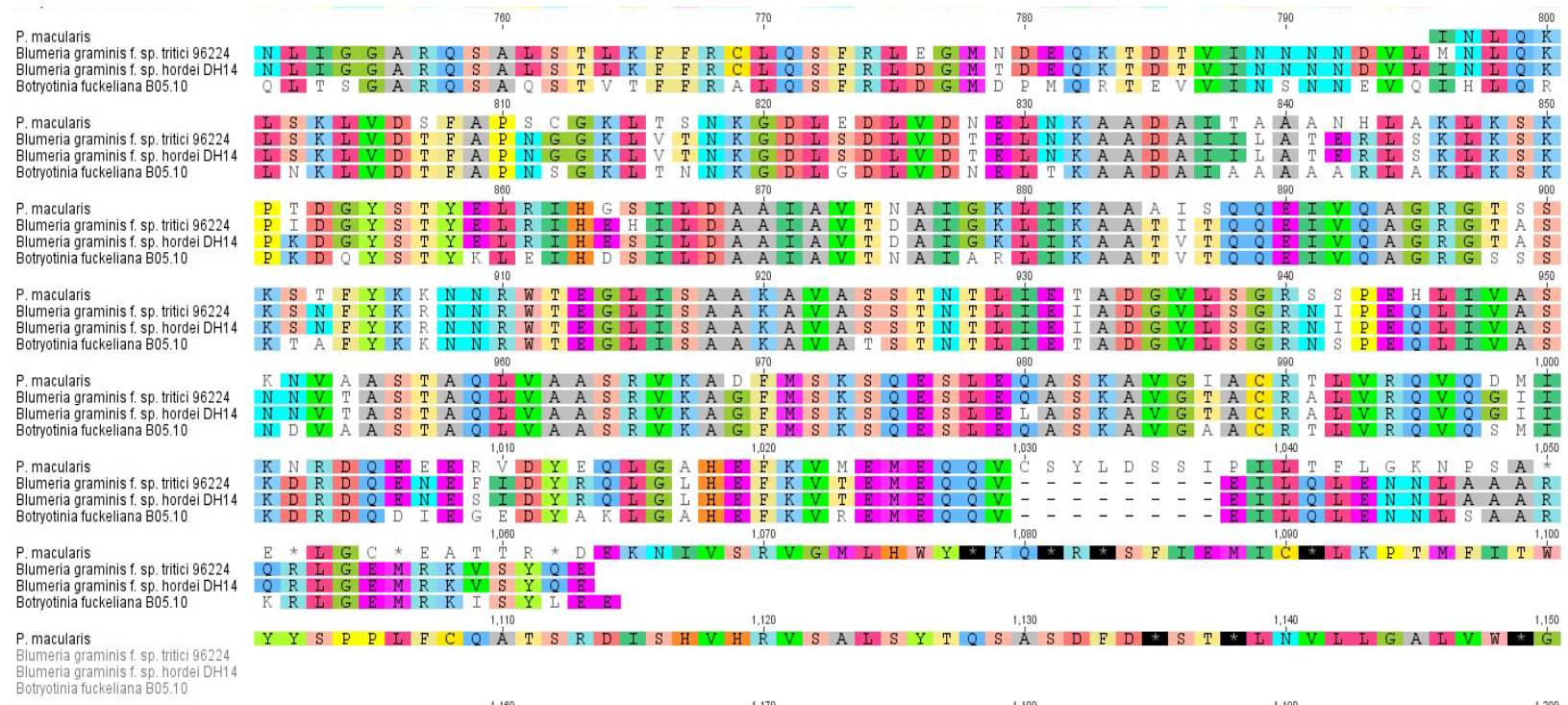
Figure 4. Alignment of *MAT1-1-3*. Nucleotide alignment (performed by Geneious version 6.0.5 [Biomatters]) of *MAT1-1-3* to demonstrate similarities between *Podosphaera macularis* (top) and *Erysiphe necator* (bottom)
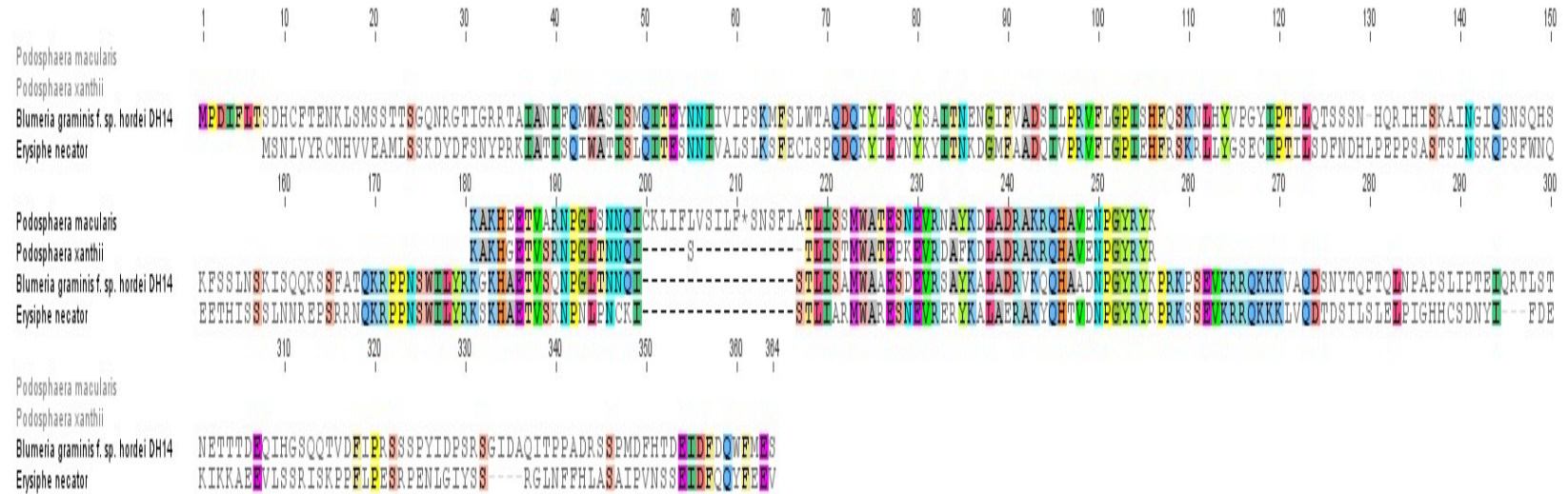
Figure 5. Alignment of *MAT1-2-1*. Amino acid alignment (performed by Geneious version 6.0.5 [Biomatters]) of the *MAT1-2-1* conserved high mobility group (HMG) domain of related species to show similarities. Top is *Podosphaera macularis,* followed by *P. xanthii, Blumeria graminis* f. sp. *hordei DH14,* and *Erysiphe necator*.

Figure 6. Alignment of *MAT1-1-1*. Amino acid alignment (performed by Geneious version 6.0.5 [Biomatters]) of *MAT1-1-1* to demonstrate similarities between closely related species. Top sequence is *Podosphaera macularis*, followed by *Erysiphe necator, Botrytonia fuckeliana BcDW1,* and *Sclerotinia borealis F-4157*.

Figure 7. Alignment of *SLA2*. Amino acid alignment (performed by Geneious version 6.0.5 [Biomatters]) of partial *SLA2*. To

demonstrate similarities between closely related species. Top sequence is *Podosphaera macularis*, followed by *Erysiphe*

necator, Blumeria graminis f. sp. *tritici* 96224, *Blumeria graminis* f. sp. *hordei* DH14, and *Botryotinia fuckeliana* B05.10
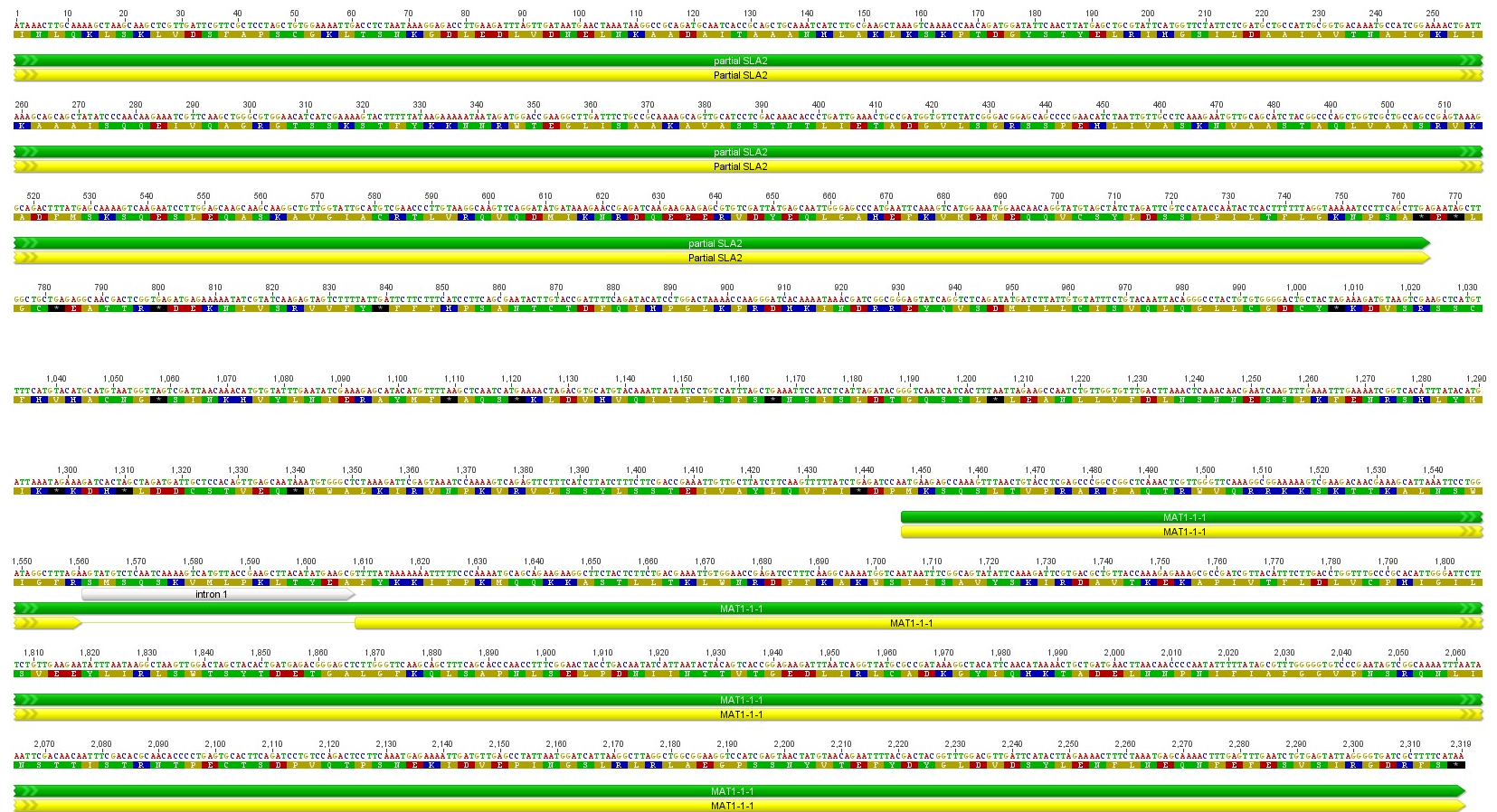
Figure 8. Partial *SLA2* connected to *MAT1-1-1* (created by Geneious version 6.0.5 [Biomatters])

Figure 9. Different modes of perennation in *Podosphaera macularis*. On the left is a newly emerged hop shoot infected with the powdery mildew fungus. These infected shoots are referred to as "flag shoots" and allow the pathogen to overwinter asexually in association with the host. On the right is a leaf with cleistothecia present (dark brown patches). Cleistothecia allow the pathogen to overwinter sexually without living host tissue. Photo at right courtesy of D. Whitener.