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Hop powdery mildew, caused by *Podosphaera macularis* Braun & Takamatsu (formerly *Sphaerotheca macularis* (Wallr.:Fr.) Lind, syn. *S. humuli* (DC.)Burrill) was not observed in Pacific Northwest hop yards until 1997, when it was discovered in Washington. Within one year, it had spread to Oregon and Idaho. This emerging disease caused severe economic losses to hop growers, due in part to the lack of resistance incorporated into commercial varieties. Despite several collection trips that increased the amount of wild hop (*Humulus lupulus* L.) germplasm available to researchers, there was no knowledge of its potential as a resource for resistance to *P. macularis*. The objectives of this study were: 1) to collect previously unrepresented native hop seed from the Southwestern United States, 2) to evaluate wild hop germplasm for resistance to *P. macularis*, and 3) to microscopically examine the progression of powdery mildew on different hop genotypes. Plant collecting expeditions were taken in September of 2002 and 2003 to obtain wild American hop germplasm (*H. l.* var. *neomexicanus*) from Colorado, Arizona, and New Mexico.

During these expeditions, 60 seedlots and 28 plant accessions were obtained. This germplasm was deposited at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Clonal Germplasm Repository (NCGR), located in Corvallis, Oregon. More than 2,100 wild (1563 North American and 107 Kazakhstani) hop seedlings, from 54 seedlots were evaluated for powdery mildew resistance. The number of resistant seedlings observed when greenhouse temperatures were >30°C was 218. The number of resistant seedlings decreased to 62 when greenhouse temperatures were <30°C. Selected resistant genotypes were evaluated for resistance under field conditions. Of these, 31 maintained resistance to foliar infections. Cones from 13 female and monoecious genotypes were evaluated for the presence of mildew; 5 genotypes from Manitoba and North Dakota and 1 genotype from Kazakhstan exhibited high cone resistance. Additional studies examined the effect of temperature on the resistance of the host. The results demonstrated that the resistance of susceptible to tolerant genotypes increased when the plant was exposed to high temperatures (>30°C) prior to inoculation. The development of *P. macularis* on several native North American and Kazakhstani hop genotypes was observed using direct light microscopy. The disease progressed slower on native genotypes than on the susceptible *H. l.* 'Symphony'. Conidia on the resistant cultivar 'Nugget' produced only the primary germ tube prior to shriveling. On 3 infected native Kazakhstani genotypes, epidermal cells at the center of the colony began to collapse at 120 h, forming a lesion that continued to radiate outward. Fungal hyphae then shriveled in response to epidermal collapse. As a result of this work, new sources of resistance to powdery mildew were identified. A high percentage of resistant seedlings were

observed in specific lots from Manitoba, Saskatchewan, and North Dakota. Resistant genotypes from North America and Kazakhstan may lead to germplasm releases for use in hop breeding programs. The partial resistance observed in wild hops suggests that multiple genes are involved. This type of resistance would be more durable than single gene resistance, which is easily overcome by the evolution of more virulent pathotypes.

Powdery Mildew (*Podosphaera macularis* Braun & Takamatsu) Resistance in Wild Hop Genetic Resources

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Dr. Kim E. Hummer aided with the experimental design and interpretation of the data in Chapter 3.

Dr. Walt F. Mahaffee assisted in the inoculations, evaluations, experimental design and interpretation of the data in Chapter 3.

James M. Oliphant was involved in the planning of the collection trips and the writing of Chapter 2.

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Chapter 1

Introduction

The Host

The common hop, *Humulus lupulus* L., is a dioecious, herbaceous perennial which produces annual bines from an over-wintering rhizome. The female inflorescences, commonly referred to as hop cones, are used in the brewing industry for flavor, and historically as a preservative. The bright yellow lupulin glands, concentrated at the base of the bracteoles, contain the resins (of which the α-acid is the most important fraction) which give beer its bitterness and the essential oils which impart aroma and flavor.

Humulus and *Cannabis* are the only two genera in the *Cannabaceae* family. The genus *Humulus* consists of three species: *H. lupulus*, a perennial bine native to Europe, Eastern Asia and North America; *H. yunannensis* Hu, a perennial bine native to southern China; and *H. japonicus* Siebold & Zuccarini, an annual bine native to Japan, Taiwan, and China, that has since been introduced in Europe and the northeastern United States.

E. Small (1978) conducted numerical taxonomic analysis of morphological features on 783 herbarium voucher specimens of *H. lupulus*. He clarified the descriptions of five botanical varieties, based on morphogeographic characters.

H. l. var. *neomexicanus* Nelson and Cockerell is distributed in western North America (Table 1.1, Fig. 1.1). This taxon possesses leaves with relatively more lobes,

deeper lobe clefts, and the greatest density of abaxial leaf glands than observed in other taxa (Small, 1978).

H. l. var. *pubescens* E. Small is found in the American Midwest (Table 1.1, Fig. 1.1). This taxon is distinguishable by numerous climbing hairs on the petioles, abundant pubescence on the abaxial midrib of the leaves, and particularly by the presence of hairs between the abaxial veins on the leaves. The leaves tend to be entire to 3-lobed and marginal serration tends to be more pronounced than in other taxa.

H. l. var. *lupulus* occurs in Europe, Asia, and Africa, and has been introduced elsewhere, especially eastern North America (Table 1.1, Fig. 1.1). This variety is distinguished from the others by possessing the fewest numbers of trichomes and glands on the abaxial leaf surface. Additionally, the number and height of the climbing hairs on the petioles are often limited, and the frequency of marginal serrations on the leaves is somewhat less than in other *Humulus* taxa.

H. l. var. *cordifolius* (Miguel) Maximowicz is found in eastern Asia, mainly in Japan (Table 1.1). This variety tends to have comparatively tall, but fewer climbing hairs than the other members of the genus.

H. l. var. *lupuloides* E. Small occurs in eastern and central North America (Table 1.1, Fig. 1.1). This variety encompasses plants of North America which, by exclusion, do not key to any of the other varieties.

In areas where the botanical varieties of *H. lupulus* are sympatric, there is potential for introgression of subspecies. Small (1980) reported pronounced intergradation between *H. l.* var. *lupuloides* and *H. l.* var. *neomexicanus* in the region of Manitoba, Canada (Fig. 1.1).

Table 1.1. Distributional range of *Humulus* L.[1]

Humulus japonicus Siebold & Zucc.
ASIA-TEMPERATE *Soviet Far East*: Russian Federation (Amur, Primorye) *China*: Anhui, Fujian, Guangdong, Guangxi, Guizhou, Hainan, Hebei, Heilongjiang, Henan, Hubei, Hunan, Jiangsu, Jiangxi, Jilin, Liaoning, Shaanxi, Shandong, Shanxi, Sichuan, Xizang, Yunnan, Zhejiang*Eastern Asia*: Japan (Hokkaido, Honshu, Kyushu, Ryukyu Islands, Shikoku), Korea, Taiwan **ASIA-TROPICAL** *Indo-China*: Vietnam *Naturalized in Europe and E. North America
Humulus yunnanensis Hu
ASIA-TEMPERATE *China*: Yunnan
Humulus lupulus.* L. var. *cordifolius (Miq.) Maxim.
ASIA-TEMPERATE *Eastern Asia*: Japan (Hokkaido, Honshu); cultivated in N. China
Humulus l.* L. var. *lupuloides E. Small
NORTH AMERICA *Canada*: New Brunswick, Newfoundland [SW], Nova Scotia, Ontario [S], Prince Edward Island, Quebec [S], St. Pierre and Miquelon, Alberta [S], Manitoba [S], Saskatchewan [S] *United States*: Connecticut, Indiana, Maine, Michigan, New Hampshire, New Jersey, New York, Ohio, Pennsylvania, Rhode Island, Vermont, Illinois, Iowa, Minnesota, Nebraska, North Dakota, South Dakota, Wisconsin, Montana, Delaware, Maryland, Virginia
Humulus l.* L. var. *lupulus
AFRICA *Northern Africa*: Morocco **ASIA-TEMPERATE** *Western Asia*: Israel, Lebanon, Syria, Turkey *Caucasus*: Armenia, Azerbaijan, Georgia, Russian Federation (Ciscaucasia, Dagestan) *Siberia*: Russian Federation (Altay, Western Siberia) *Soviet Middle Asia*: Kyrgyzstan *China*: Xinjiang **EUROPE** *Northern Europe*: Denmark, Finland, Ireland, Norway, Sweden, United Kingdom *Middle Europe*: Austria, Belgium, Czechoslovakia, Hungary, Netherlands, Poland, Switzerland *East Europe*: Belarus, Russian Federation (European part), Ukraine (incl. Krym) *Southeastern Europe*: Albania, Bulgaria, Greece, Italy, Romania, Yugoslavia *Southwestern Europe*: France, Portugal, Spain *Widely naturalized and cultivated, especially in E. North America
Humulus l.* L. var. *neomexicanus A. Nelson & Cockerell
NORTH AMERICA *Canada*: Manitoba [SW], Saskatchewan [SE] *United States*: North Dakota, South Dakota [W], Wisconsin, Colorado, Montana, Wyoming, New Mexico, Texas, Arizona, California [N], Nevada, Utah *Mexico*: Chihuahua
Humulus l.* L. var. *pubescens E. Small
NORTH AMERICA *United States*: Indiana, Michigan, Ohio, Pennsylvania, Illinois, Iowa, Kansas [E], Minnesota [SE], Missouri, Nebraska [E], Wisconsin, Maryland, North Carolina [N], Virginia

[1]GRIN, 2005.

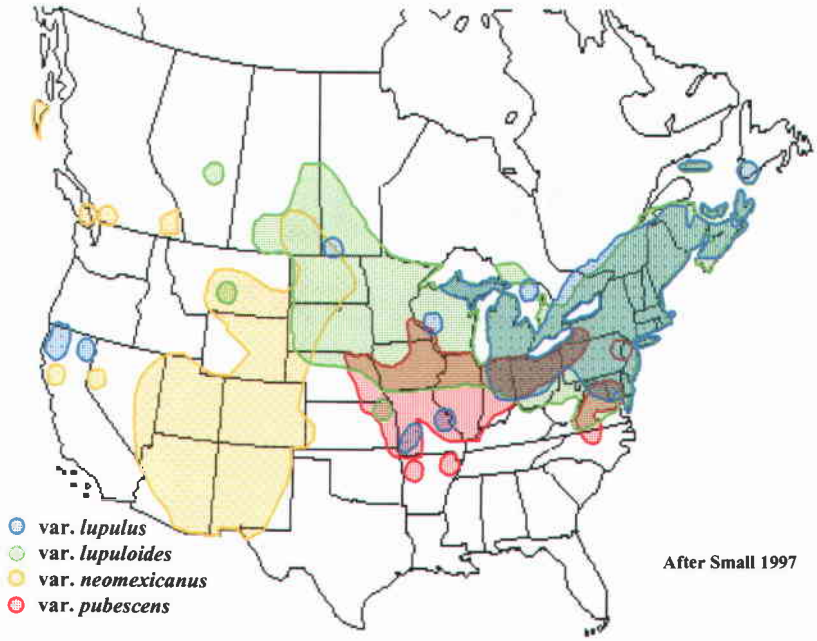

Fig. 1.1. Distribution of *Humulus lupulus* in North America.

Native North American hop germplasm was first used for hop breeding by Professor E. S. Salmon at Wye College, England. In 1916, cuttings collected by Professor W. T. Macoun from a wild hop growing near Morden, Manitoba, Canada were sent to Wye College. From these cuttings, Salmon selected a plant referred to as 'BB1'. Although 'BB1' died in 1918, a seedling offspring, 'C9a', "attracted attention in 1925 by the extraordinary richness of its cones, which were so full of condition that on being opened and rubbed they became greasy or buttery to the touch" (Salmon, 1934). The exceptionally high preservative power of 'C9a' gave it "the distinction of being the richest hop in the world" (Salmon, 1934). 'C9a' was released in 1934 as *H. l.* 'Brewer's Gold' (Wild Manitoba 'BB1' x Open Pollinated). Salmon (1934) when discussing the disease resistance of 'Brewer's Gold' wrote that "No attacks of

mould [powdery mildew] have occurred either at Wye or at East Malling." 'Brewer's Gold' was a major hop variety, particularly in Oregon, until it was discontinued from commercial production in 1985, after the advent of super-alpha hops which had higher alpha-acid content and improved storage stability.

Many of the high-alpha hops used today are descendents of 'Brewer's Gold'. Cultivars with 'Brewer's Gold' in their pedigree include *H. l.* cvs. Ahil, Apolon, Atlas, Chinook (¼ Brewers Gold and another ¼ from a wild hop from Utah) (Kenny and Zimmerman, 1986), Crystal, Galena, Horizon, Nugget, Olympic, OT 48, Pride of Kent, and USDA 21055.

H. l. 'Bullion' also originated from the native North American hop, 'BB1'. In 1919, the seedling was raised from open pollinated seed collected on the female 'BB1' in the hop nursery at Wye College, by Salmon (Salmon, 1938). A major hop variety since the mid-1940's, 'Bullion' was also discontinued from commercial United States production in 1985, after the advent of super-alpha hops.

H. l. 'Cluster', the oldest United States cultivar, is a diploid seeded hop, derived from native *Humulus*. The pedigree of 'Cluster' is likely the result of crosses between English varieties brought to the new world by colonists and a wild male American hop. At the turn of the century, more than 96% of the American hops grown were the cultivar 'Cluster' (Haunold *et al.*, 1985). 'Cluster' is a mid-range alpha variety that is not resistant to powdery mildew.

H. l. 'Comet' was another early selection of a North American wild hop. 'Comet' resulted from a cross made in 1961, between a wild male hop collected from Logan Canyon, Utah, and a seedling of the English hop *H. l.* 'Sunshine'

(Zimmermann *et al.*, 1975). In 1980, 'Comet' comprised 1% of hop production in the United States, but its' production declined by 1981 due to the release of super-alpha hops.

Other cultivars with wild hops in the pedigree include *H. l.* cvs. Density (the maternal parent, Keysworth's Midseason, was derived from *H. l.* var. *neomexicanus*), Hallertaur Magnum, Hallertaur Tradition, Hersbrucker Pure, Hüller Anfang, Spalter Select, W45 (wild hop from Thüringen), and Wye Target.

A bottleneck of genetic material is currently used in the development of new cultivars (Henning, *et al.*, 2004; Jakse *et al.*, 2004; Seefelder, *et al.*, 2000; Sustar-Vozlic and Javornik, 1999; Pillay, and Kenny, 1996). Limited genetic diversity in parental lines leads to high levels of inbreeding, loss of genetic variability, and reduced potential for continued improvement. Inbreeding depression reduces vigor and increases undesirable traits. This narrow genetic gain can also give wide susceptibility to environmental factors, pests, and diseases.

Hops have been grown commercially in the Pacific Northwest since the 1850's (Barth *et al.*, 1994). Hop powdery mildew, caused by *Podosphaera macularis* Braun & Takamatsu (formerly *Sphaerotheca macularis* (Wallr.:Fr.) Lind syn. *S. humuli* (DC.)Burrill) was unknown in the field of this area until 1997, when it was observed in a commercial hop yard in the Yakima Valley of Washington State (Ocamb *et al.*, 1999). Approximately 800 of 12,000 hectares in production were destroyed in 1997, due to hop powdery mildew (Ocamb *et al.*, 1999). More than 1,200 hectares of highly susceptible hop varieties were replaced, resulting in an estimated loss of $9.5 million

in combined production and establishment costs (Turechek *et al.*, 2001). In 1998, hop powdery mildew was discovered in Idaho and the Willamette Valley of Oregon.

Powdery mildew and its control is estimated to have cost Pacific Northwest hop growers over $30 million in 1999 and 2000, or about 15% of their total crop revenue (Turechek *et al.*, 2001). Management was accomplished through 10 to 13 fungicide applications at a cost of approximately $1000/hectare.

The Pathogen

Hop powdery mildew, caused by *Podosphaera macularis*, is the oldest known fungal disease of the cultivated hop (*H. lupulus*) (Royle, 1978). Since before 1700, powdery mildew has been the most serious disease of hops in certain areas of England (Royle, 1978). By the late 1800's, powdery mildew had spread to each of the hop growing areas in England, as well as Western Europe and Russia. At that time, mild attacks were recorded in Maine, California, Alabama, and Wyoming in the United States (Blodgett, 1913). In 1909, severe mildew outbreaks occurred in New York, causing the total failure of many hop yards. The most destructive year, 1912, had an estimated loss of revenue of $333,000. Since that time, the main hop growing region in the United States shifted to the Pacific Northwest.

Podosphaera macularis is an obligate biotroph in the Phylum Ascomycota (Family: Erysiphaceae). It survives in absence of its host in dormant structures, called cleistothecia, which appear as spherical pinhead size, 58 to 120μm (Blodgett, 1913), brown to black spots on infected tissue. Each cleistothecium encloses a single ascus, which contains eight ascospores (formed by meiosis). *P. macularis* is heterothallic

with 2 known mating types, nominally called positive and negative. In response to complex factors, most likely temperature rather than day length or host status, cleistothecia are produced around the time of harvest. They are shed to the soil surface and plant debris during harvest. Although *P. macularis* may over-winter as mycelium in buds, cleistothecia ensure survival of the fungus by being resistant to severe frosts (below -20 °C) and fungicide application. To date, there is no confirmed presence of cleistothecia in the Pacific Northwestern United States.

Liyanage and Royle (1976) observed cleistothecia production over three years in England. They observed that during the winter, cleistothecia mature in two periods, ending in November and in March/April. Many then differentiate into eight, well-defined ascospores. After this apparent obligate dormancy, ascospore discharge coincides with the growth of new foliage helping to ensure pathogen survival (Royle, 1978). Commonly, 30 to 50% of cleistothecia have dehisced by the end of April (Royle, 1978). Although the precise conditions necessary for ascospore discharge is not known, cleistothecia will dehisce only when wetted, so presumably rain or dew is required for ascospore release in the field (Royle, 1978).

During the growing season, *P. macularis* is spread asexually by conidia. The pathogen exists only on the plant surface and subsequent spread involves re-infection of the host. The spores are asexual and so are identical to the mating type and strain of the parent colony. Conidia are dispersed by wind or rain splash to other leaves. Salmon (1907) reported that *P. macularis* appears to be genus specific and did not infect other plant species examined. His cross-infection studies, however, did not include the most closely related taxa, *Cannabis*. *Cannibis* could be an alternate host

since numerous other Erysiphaceae species infect most members of a plant family (e.g. *Erysiphe cichoracearum* infects begonia, chrysanthemum, cucurbits, dahlia and zinnia; *E. polygoni* infects legumes, beets, crucifers and cucurbits; *Podosphaera oxyacanthae* infects apricot, cherry, peach and plum.)

Unlike cleistothecia, which require water to dehisce, conidia are able to germinate under dry conditions. Infection is actually encouraged by a dry leaf surface. High levels of infection can also be obtained by inoculating hop leaves with an aqueous suspension of conidia (Royle, 1978), as long as leaves are air-dried within 1 h of inoculation to prevent lysis of conidia (Mahaffee, *et al.,* 2003). Sporulation is favored under drier conditions and mycelial development is favored by more humid conditions (Darby, 1998).

On a square millimeter of mildewed leaf surface, Blodgett (1913) counted 440 conidiophores, averaging 8 conidia each. This would mean 285,000 on a square inch of leaf surface covered with mildew, or 2,280,000 conidia. Turechek *et al.* (2001) reported that the latent period of *P. macularis* is 5 days at 18 to 27°C and 10 days at 12 to 15°C. Given the rapid latent period, and a hop growing season of approximately 6 months, it is possible that *P. macularis* can have 25 to 40 generations a growing season. This fecundity accounts for the rapid spread of the disease throughout a hop yard, particularly in the hop growing regions of the Pacific Northwestern United States, where the warm, dry conditions favor the disease.

Turechek *et al.* (2001) examined the effect of constant temperature on disease development. Hop powdery mildew developed in the range of 12 to 27°C, with 27°C having a significantly lower infection frequency than the other temperatures. Later,

Mahaffee, *et al.* (2003), determined that 28°C was the upper limit for infection. The optimal temperature for infection, growth and sporulation was 18°C.

The infection severity of *P. macularis* is highly sensitive to exposure to high temperatures (Mahaffee *et al.*, 2003). Exposure of hop plants to 30°C for as little as 2 h at the time of infection reduced the infection severity by 50% or more. Exposure of plants to temperatures >39°C, which frequently occurs in the hop growing regions of the Pacific Northwest, appeared to cause colony death.

The Disease

Hop powdery mildew is characterized by the appearance of spots or patches of white to grayish 'powdery' growth on plant tissues. As the fungus grows, the circumferences of the spots enlarge, and eventually coalesce to form a dense mat of mycelia. Powdery mildew is commonly observed on the adaxial surfaces of the leaves, but it also affects the abaxial surfaces, young shoots and stems, buds and flowers.

Hop leaves have ontogenic resistance to powdery mildew with newly unfolded leaves being the most susceptible and leaves older than 15 days do not become infected (Turechek *et al.*, 2001). *P. macularis* infections on leaves and stems do not generally result in serious economic damage, but serve as a source of inoculum for flower and cone infections that can cause serious economic damage. Infection can cause death of the flower or deformation of the cone, resulting in yield loss. Severely deformed cones will not survive the cleaning process during harvest. Infection of the maturing cones will often result in discoloration and loss of quality rather than loss of

weight of the crop (Darby, 1998). Infection above 2% causes unacceptable discoloration, and α-acid levels are reduced when infection levels are above 5% (Darby, 1998). Bitter acid analyses of the varieties *H. l.* cvs. Saaz, Magnum and Premiant showed that cones damaged by powdery mildew have 12 to 25% lower α-acid content (Krofta and Nesvadba, 2003).

Resistance

Resistance to *P. macularis* was first recorded by E. S. Salmon (1917a). In his hop breeding program he examined the inheritance of various forms of resistance (Salmon, 1917a, 1919a, b, 1920, 1921, 1927). Two types of resistance were described: 1) immunity, in which nine seedlings from seed of a 'wild hop' obtained from Italy, and a form of hop with yellow leaves, called 'Golden Hop' remained uninfected despite heavy inoculum levels in a greenhouse, and 2) 'semi-immunity', in which leaves of plants from Italy responded to infection by producing blisters, as in a susceptible reaction (Salmon, 1917b), except that only a weak, faintly white mycelium developed which then died, leaving small patches of dead epidermal cells (Salmon, 1919a).

P. macularis appears to have several pathogenic strains that appear to correspond to races in a classic gene-for-gene relationship with host resistance (Darby, 2001). Seven resistance genes have been suggested for which there are corresponding virulence genes in the pathogen. A series of crosses between parents thought to be carrying the relevant resistance gene and a susceptible parent (seedlings of 'Northern

Brewer') indicated that resistance is controlled by a single gene in each progeny (Darby, 2001).

Expression of the blister gene (R_B), found in *H. l.* cvs. Yeoman and Wye Challenger, is temperature dependent and variable (Darby, 1998). Blisters appear to be an unusual reaction among powdery mildews and are suggestive of a localized hormonal imbalance (Royle, 1978). The R_B gene is genetically associated with production of the essential oil selinene and appears to be lethal when homozygous (a seed with 2 copies of the gene will not germinate) (Darby, 1998).

The R_1 gene, initially confused with the R_3 gene, was incorporated into the variety 'Zenith', and remained effective until 1980. When the new fungal strain first appeared, it could only weakly overcome the R_1 gene. More aggressive strains continued to develop, and by 1983, 'Zenith' was fully susceptible.

The R_2 gene has been characterized in *H. l.* 'Wye Target'. The source of this gene is from a powdery mildew and verticillium wilt (*Verticillium albo-atrum*) resistant, open-pollinated, *H. l.* var. *neomexicanus* seedling acquired from the United States. It is the basis of resistance breeding in both the United Kingdom and German hop improvement programs. Expression of this gene in the greenhouse is often incomplete (Darby, 1998). The resistance of 'Wye Target' has remained stable in the field since its release in 1973, despite three reports of infected plants in 1979 (Neve, 1991). Between 1996 and 1997, a new strain which could overcome this resistance gene appeared.

The R_3 gene has been documented in 'Wye Challenger'. Apparently the corresponding virulence gene was present widely within the United Kingdom

pathogen population and there was rapid selection for this strain as soon as the variety entered commercial production.

The Russian variety 'Serebrianka' possesses the R_4 gene. The R_5 gene is found in the variety 'Early Choice'. It confers resistance rather than immunity. Tests carried out at Wye College in 1990 indicated that 'Cascade' showed identical reactions to a range of isolates. In 1998, *P. macularis* isolates able to overcome the R_5 gene existed in low frequency in the mildew population of the United Kingdom.

The R_6 gene is characterized in 'Nugget'. It has been overcome by corresponding pathogenic strains in Germany, Belgium, France, and the United Kingdom. At Wye, the strains are less frequent than others and the variety only shows occasional sporulating lesions. 'Nugget' continues to be a resistant variety widely grown in the Pacific Northwest.

In addition to the major resistance genes, a polygenic form of resistance to powdery mildews in general, also exists (Bushnell, 2002). This partial resistance is general or race-nonspecific and has pleiotropic effects. In some cases, penetration of the fungus is blocked, but more often the size of haustoria is reduced, limiting the growth and sporulation of colonies. Reduced colony development usually occurs in the absence of hypersensitive responses visible to the naked eye (Bushnell, 2002). Limited fungal growth may be due to the reduced efficiency of haustoria to obtain nutrients from the host. Interest has been shown in genotypes with high levels of partial resistance under polygenic control on the assumption that such resistance may be more durable (Johnson, 1984). Often the resistance is difficult to measure and no accurate estimate of the number of genes involved can be made.

Darby *et al.* (1989) found that heritability of partial resistance was high from male parents, indicating the possibility of such a selection within a breeding program. On its own, such resistance could allow for the disease to be controlled easily with only a few fungicide applications. The best strategy may be to combine partial resistance with resistance controlled by major genes in a single variety (Darby *et al.*, 1989). This would reduce the selection potential for a virulent strain that could overcome the major gene resistance, and should this happen, the polygenic resistance would still be operative (Neve and Darby, 1982).

One class I chitinase gene has been sequenced and characterized from genomic DNA isolated from leaves of the hop variety 'Zenith' (Henning and Moore, 1999) which possesses three major resistance genes (RB, R1 and R3) (Darby *et al.* 1989). The cell walls of many fungal hyphae are strengthened with chitin, a polysaccharide analogous in chemical structure to cellulose, consisting of units of a glucose derivative (*N* -acetyl- d -glucosamine) joined to form long, unbranched chains. Like cellulose, chitin contributes to the strength and protection of the fungus. Plants possessing the enzyme chitinase are able to degrade chitin. The production of chitinase, which can break down fungal cell walls, may be an important defense response by the plant. In some crops, fungal growth is stopped by lysis of the hyphal tip (Schlumbaum *et al.*, 1986). Patzak (2003) found the presence of this chitinase gene in 68 international hop cultivars.

Royle (1978) discussed the interactions between eight isolates of powdery mildew and several hop genotypes. The observations, made by Liyanage (1973), were of the fungus on leaf discs kept at optimum temperatures for infection (17 ± 2° C). In

compatible interactions, in which the plant is susceptible, the primary germ tube is produced 6 h after inoculation. The germ tube penetrates the host and establishes a haustorium within 12 to15 h. After 48 h, the initial germ tube becomes a branching hypha and up to 3 more germ tubes arise. Conidiophore initials appear after 96 h and the first conidia are visible shortly afterwards. With no apparent changes on the host surface, sporulation increases 5 to7 days after inoculation.

Fungal development is not different between compatible and incompatible (isolates allowing expression of R-gene resistance) reactions up to emergence of the first germ tube and attempted host penetration, 12 to15 h after inoculation. The interaction between the fungus and the host cytoplasm differentiates the two reactions (Royle, 1978). In hop genotypes containing the R_B gene (for 'resistant blister'), mycelial growth is noticeably hindered within 30 h, as compared to that on a susceptible host. Typically, only one or two germ tubes emerge, although up to four may be produced. Hypersensitivity, or epidermal cell death, is relatively delayed until after the formation of haustoria, only a proportion of which remain functional (Liyanage, 1973). Sporulation is comparatively weak with fewer conidiophores being produced with shorter conidial chains. Epidermal cells collapse, even in advance of the hyphae.

In genotypes expressing the R_1 gene, epidermal cells reacted hypersensitively immediately after penetration by the fungus (Liyanage, 1973). Haustoria were degenerated allowing no colony development.

In genotypes expressing the R_2 gene, such as 'Wye Target', the pathogen fails to develop beyond the primary germ tube (Liyanage, 1973). An immediate

hypersensitivity resulted as a response to attempted penetration; no haustoria or hyphal development is observed. By 96 h, the germinated conidium has collapsed.

In susceptible genotypes, as many as three haustoria are produced, although one is the most common (Royle, 1978). The haustoria consist of a nucleated body surrounded by a finely granular matrix bound by the host plasmalemma. The haustorial body produces lobed outgrowths which extend its surface within the sheath matrix. The cytoplasm of the infected epidermal cell remains organized as do the contents of the adjacent cells of the palisade mesophyll.

With incompatible reactions, haustoria differentiate and then lyse, differentiate abnormally, or are not produced, depending upon the particular pathotype : host genotype combination (Royle, 1978). Host genotypes with the R_B gene typically allow the first haustoria, produced 12 to15 h after inoculation, to form normally. By 21 h, these have begun to lyse, after supporting some hyphal growth. Subsequent haustoria act similarly or are malformed to begin with, without clearly differentiated organelles. Malformed or dying haustoria are associated with epidermal and palisade cells which have reacted hypersensitively to the pathogen. In immune hosts, haustoria are either malformed or never produced, as in genotypes with the R_2 gene. In the latter case, the epidermal cells react violently to attempted penetration.

Godwin *et al.* (1987) studied the interactions between an isolate of the 'Zenith' pathotype (widespread in the United Kingdom) and the hop cultivars *H. l.* 'Northern Brewer' (susceptible) and 'Wye Target' (R_2 gene). Total germ tube and hyphal length were markedly less on 'Wye Target' than on 'Northern Brewer'. Conidia on 'Northern Brewer' usually produced at least two germ tubes within 48 h of

inoculation, while on 'Wye Target' typically a single, short germ tube was produced. Slightly fewer sporelings formed haustoria initials in 'Wye Target' and growth of the fungus was usually restricted soon afterwards. Haustoria formed in 'Wye Target' leaves lacked the prominent lobes developed by haustoria in susceptible leaves. A difference in the frequency with which a mesophyll cell reaction occurred beneath sporelings was also apparent between the two genotypes.

Granulation of penetrated epidermal cells occurred rapidly in the resistant variety and by 48 h was noted in most cells containing a haustoria initial or haustorium. Granulation rarely occurred in the penetrated cells of 'Northern Brewer'. Histochemical staining techniques identified lignin-like material in the granular contents of affected epidermal and palisade mesophyll cells, as well as in epidermal cell walls. Thick deposits of callose-like material (β-1,3-linked glucans) and a marked browning reaction were often seen in palisade mesophyll cells adjacent to necrotic epidermal cells containing dead or dying haustoria. Callose-like material was also deposited at sites of fungal penetration, forming a collar around the haustorial neck in both resistant and susceptible leaves. Godwin *et al.* (1987) concluded that the hypersensitive death of penetrated epidermal cells associated with widespread callose deposition and lignification in penetrated epidermal and underlying palisade mesophyll cells are the mechanisms underlying resistance in 'Wye Target'.

The recent appearance of powdery mildew in the Pacific Northwestern United States and the limited sources of resistance emphasized the need to increase the genetic diversity within hop germplasm collections. During the past 23 years, 15 USDA and privately-sponsored plant collecting expeditions have donated hop plant

material to the National Clonal Germplasm Repository (NCGR), in Corvallis, Oregon (Hummer, 2005). Most accessions are stored as seed. Resistance in this material would provide a valuable new genetic resource. The objectives of this thesis were to evaluate this new germplasm of wild collected *Humulus* for resistance to powdery mildew, to study the development of the fungus on different genotypes, and to collect unrepresented native hop germplasm from the Southwestern United States.

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Chapter 2

Plant Explorations to collect the Native American hop, *Humulus lupulus* var. *neomexicanus* Nelson and Cockerell, from Arizona, Colorado, and New Mexico

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Summary

Plant collecting expeditions were taken on 9 to 20 September 2002 and 8 to 19 September 2003 to obtain genetic resources of wild American hop germplasm (*Humulus lupulus* var. *neomexicanus*) from Colorado, Arizona, and New Mexico. Much variation in morphology and aroma was observed in most populations. *Humulus* distribution in the Rocky Mountains is scattered and uncommon. While herbarium locality data was invaluable to locate populations, no clear predictors of potential habitat based on associated species, topography, or even proximity to water were evident after our extensive survey. *Humulus* was much more fragmented on east slope than the west slope of the Rocky Mountains. The drier climate and loss of habitat through human development of the east slope may be factors contributing to this observation. *Humulus* appears to have an opportunistic, fragmented distribution pattern and may be undergoing range expansion or local extinction. In 2002, 35 populations of *H. l.* var. *neomexicanus* from 12 major drainage basins in Colorado and New Mexico were sampled resulting in 54 seed accessions (totaling 625,000 seeds) and 16 plant accessions. Herbarium specimens were collected from 9 significant new localities to be distributed to major regional herbaria. In 2003, 13 populations of *H. l.* var. *neomexicanus* were sampled in Arizona and New Mexico resulting in six seed accessions (totaling 6000 seeds) and 12 plant accessions. The seed and plant accessions were deposited at the United States Department of Agriculture (USDA) Agricultural Research Service (ARS) National Clonal Germplasm Repository (NCGR) Corvallis, Oregon, and are available to researchers upon request.

Introduction

Native North American hops, *Humulus lupulus* L. vars., have historically been poorly represented in *Humulus* germplasm or breeder collections. This seems surprising, because there are 3 recognized botanical varieties of *Humulus lupulus* that are indigenous to North America and show much geographic variation over a vast range (Small, 1997). In the last 20 years, more than 10 American expeditions have collected wild *Humulus lupulus* material (reviewed in Hummer, 2005).

Native North American *Humulus lupulus* has the potential for unique traits that may be invaluable to hop breeding programs, such as novel growth forms, e.g. dwarf, non-vining, low chill genotypes, secondary chemistries, and resistance to hop powdery mildew (*Podosphaera macularis* Braun & Takamatsu). Small (1980) outlined the pedigree of hop cultivars that have notable resistance to hop powdery mildew, and found that they all have *H. l.* var. *lupuloides* E. Small in their background. In previous studies, seedlings of *H. l.* var. *lupuloides* and *H. l.* var. *pubescens* E. Small have exhibited resistance to powdery mildew (Chapter 3). In addition, native North American *Humulus* could have the potential for other valuable agronomic traits such as aphid and virus resistance (Hampton *et al.*, 2001).

H. lupulus is a widespread dioecious, long-lived perennial that is wind pollinated (Small, 1997). *H. lupulus* has an obligate outcrossing mating system that is typical of other long-lived plants (Ledig, 1986). The geographic pattern of genetic variation in widespread outcrossing species requires a sampling strategy that attempts to disperse the sample points as much as possible throughout the entire range of the species (Millar and Libby, 1991). Much of the current range of *H. lupulus*, including

all of *H. l. lupuloides*, was either glaciated or cold mixed conifer forest as recent as 10,000 years ago (Delcourt and Delcourt, 1993). This implies that *H. lupulus* has undergone a rapid expansion of range from southern populations during the Holocene period and is most likely continuing to expand its range (Delcourt and Delcourt, 1993, MacDonald and Cwynar, 1985). Only southern areas of the current distribution of *H. lupulus* are in regions that were unglaciated during the Pleistocene (Small, 1997, Pielou, 1991, Delcourt and Delcourt, 1993). This potential historical impact on genetic variation suggests: 1) southern populations should have the greatest diversity, both among and within populations (Critchfield, 1984); 2) genetic diversity within populations decreases clinally northwards (Millar and Libby, 1991); and 3) adaptive and physiological traits vary clinally from south to north (*e.g.* Cwynar and MacDonald, 1987).

Considering the geographic distribution of *H. lupulus* in the context of Quaternary vegetation history suggests that populations of *H. l.* var. *neomexicanus* in Colorado, New Mexico, Utah, Arizona, Nevada, Wyoming, and Mexico (and to a lesser extent the populations of *H. l.* var. *pubescens* in the eastern United States) should harbor significant genetic diversity that would have accumulated during repeated interglacial introgression and glacial isolation events as the range of *H. lupulus* repeatedly expanded and contracted (Critchfield, 1984).

Recent collecting trips have attempted to obtain viable germplasm from the indigenous range of *H. lupulus* in North America. In 1999 and 2001, seed of *H. l.* var. *lupuloides* was collected in North Dakota, as well as Manitoba and Saskatchewan, Canada, while seed of *H. l.* var. *pubescens* was collected from Missouri in 1999.

The objective of the collection trips in 2002 and 2003 was to obtain genetic resources of *H. l.* var. *neomexicanus*. In 2002, seeds and rhizomes of wild hops were collected along major river drainages of the southern Rocky Mountain region of Colorado and New Mexico. An additional trip was made in 2003 to Arizona and New Mexico to collect from fragmented southern populations occurring on isolated montane islands of habitat.

Methods

Herbarium label data from *Humulus* collection records were obtained from the major regional herbaria as well as the National Herbarium (US). The regional herbaria are the Rocky Mountain Herbarium (RM), University of Wyoming, Laramie, WY; University of Colorado Museum Herbarium (COLO), Boulder, CO; Colorado State University Herbarium (CS), Ft. Collins, CO; Herbarium (ASC), Adams State College Alamosa, CO; University of New Mexico Herbarium (UNM), Albuquerque, NM; and the Biology Herbarium (NMC), New Mexico State University, Las Cruces, NM. The locality data from these records was then mapped to 7 ½ minute topographic maps.

During 9 to 20 September 2002 (Appendix 2.1), a trip was made to collect *H. l.* var. *neomexicanus* from the southern Rocky Mountain region of Colorado and New Mexico. The southern Rocky Mountains were divided into major watersheds on both sides of the Continental Divide (Tables 2.1 & 2.2). Within each of the watersheds an attempt was made to sample 3 to 6 populations. At each population there was an effort to sample 5 subsites of 1 to 10 individuals separated by at least 100m. The

vining growth habit of *Humulus* and its tendency to layer and root at a distance from the original crown made determination of unique clones in the field difficult. Collecting at different subsites increased the confidence that unique clones were sampled. Seed from clones were collected when possible. Cuttings or rootstocks were collected if seed set was sparse or lacking. When plants were collected an effort was made to sample both male and female clones.

A second collection trip was made from 8 to 19 September 2003, to Arizona and New Mexico (Table 2.3, Appendix 2.2). Potential *Humulus* habitat is limited in this region, generally occurring on isolated mountain ranges. An attempt was made to find *Humulus* in these 'montane islands' using locality data obtained from herbarium collection records. The sampling strategy followed the same methods as the first trip.

Results and Discussion

2002

Humulus lupulus var. *neomexicanus* was collected from 35 populations found throughout the 12 major drainage basins that occur in Colorado and New Mexico. These collections resulted in 54 seed and 16 plant accessions (Tables 2.1 and 2.2, Figs. 2.1 and 2.2, Appendix 2.1). The seed lots total about 625,000 seeds. The seed and plant accessions were deposited at the USDA ARS NCGR in Corvallis, Oregon.

Herbarium specimens were collected from 9 populations representing significant new localities. These specimens will be distributed to the following herbaria: CS, CO, NM, RM, US.

Much variation in morphology and aroma was observed in most populations. Powdery mildew was observed on plants in one location, Silver Plume, Colorado (Fig. 2.1). *Humulus* distribution in the Rocky Mountains is scattered and uncommon. While herbarium locality data was invaluable to locate populations, there appeared to be no relationship between the presence of *Humulus* and associated species topography, or even proximity to water after this extensive survey. *Humulus* appears to have an opportunistic, fragmented distribution pattern and may be undergoing range expansion or local extinction. *Humulus* was much more fragmented on east slope than the west slope. The drier climate and loss of habitat through human development of the east slope may be factors contributing to this observation (West, 1988).

Generally, when a *Humulus* site was located, plant material was collected from all possible individuals. Due to the fragmented distribution pattern, it was not possible to always sample subsites as had been originally planned.

2003

Thirteen populations of *H. l.* var. *neomexicanus* from Arizona and New Mexico were sampled resulting in 6 seed and 12 plant accessions (Table 2.3, Fig. 2.2, Appendix 2.2). The seed lots total about 6000 seeds. At most localities, only a single individual was found. This was usually an un-pollinated female. Powdery mildew was not observed on any wild *Humulus* in Arizona and New Mexico.

Using herbarium label data from university specimens, 21 localities were visited, where *Humulus* had previously been collected. The focus was on the southern isolated *Humulus* localities. *Humulus* was found at only 11 of these localities. Two

additional populations were discovered en route. In the 10 localities where *Humulus* was not found, the local extinction was possibly caused by degraded habitat or vegetation change. The increase in juniper (*Juniperus scopulorum* Sarg.) cover throughout southwestern United States has been suggested as a probable cause for the recent (last 150 years) reduction in surface stream flow (West, 1988). The combination of overgrazing and juniper growth may be causing local extinction of *Humulus*, especially at marginal sites. The most stable habitat for *Humulus* appeared to be a perennial stream with a well developed cover of willow (*Salix spp.*). Another, alternative situation is at the base of rocks in a riparian corridor where the stream is at a distance or seasonal. It appears that *Humulus* in Arizona and New Mexico is less abundant and, is undergoing much local extinction, since many of the previous collections 50 to 100 years ago are no longer extant. This suggests the genetic diversity in *Humulus* from these southern populations is threatened and may continue to be eroded through local extinction.

Table 2.1. Summary data of *H. l.* var. *neomexicanus* collected in East slope watersheds.

Watershed	# of Subsites	Form	Elevation (m)	Date
East Slope Continental Divide:				
South Platte R.				
Coal Cr., CO	1	Seed	1829	9/10/2002
El Dorado Springs, CO	4	Seed	1834	9/10/2002
Redstone Cr., CO	2	Seed	1768	9/11/2002
Sedalia, CO	1	Seed	1779	9/20/2002
Silver Plume, CO	1	Seed	2791	9/15/2002
Arkansas R.				
Nathrop, CO	1	Seed	2348	9/16/2002
Poncha Cr., CO	1	Seed	2532	9/16/2002
Phantom Canyon, CO	2	Seed/Plants	1980	9/20/2002
Purgatoire R.				
Cordova Plaza, CO	1	Seed	2070	9/19/2002
Wooton, CO	1	Seed	2201	9/19/2002
Canadian R.				
Cimarron R., NM	2	Seed	2480	9/19/2002
Manuelitas, NM	1	Seed	2176	9/19/2002
Pecos R.				
Pecos, NM	2	Seed/Plants	2341	9/19/2002
Rio Grande				
Las Huertas, NM	1	Seed	2149	9/18/2002
Rock Cr., CO	2	Seed	2507	9/17/2002
Sangre de Cristo, CO	2	Seed	2475	9/17/2002
Wagon Wheel Gap, CO	1	Seed	2537	9/17/2002

Table 2.2. Summary data of *H. l.* var. *neomexicanus* collected in West slope watersheds.

Watershed	# of Subsites	Form	Elevation (m)	Date
West Slope Continental Divide:				
Green & Yampa R.				
Beaver Cr., CO	1	Seed	1733	9/13/2002
Buford, CO	1	Seed	2157	9/13/2002
Deer Gulch, CO	2	Seed/Plants	2129	9/14/2002
Miller Cr., CO	1	Seed	2192	9/13/2002
South Fork Canyon, CO	1	Seed	2338	9/13/2002
White R.				
Axial, CO	4	Seed	2005	9/12/2002
Hayden, CO	4	Seed	1978	9/12/2002
Milner, CO	1	Seed	2063	9/12/2002
Colorado R.				
Aspen, CO	1	Seed	2437	9/15/2002
E. Rifle Cr., CO	2	Seed	1948	9/14/2002
Sweetwater Lk., CO	3	Seed	2363	9/14/2002
Gunnison R.				
Cochetopa Canyon, CO	1	Seed	2439	9/16/2002
Tomichi Cr., CO	1	Seed	2518	9/16/2002
Willow Cr., CO	1	Seed/Plants	2350	9/16/2002
Dolores/San Miguel R.				
Delores R., CO	1	Seed	2265	9/18/2002
Leopard Cr., CO	2	Seed	2264	9/18/2002
San Juan/ Animas R.				
Cherry Gulch, CO	1	Seed	2164	9/18/2002
Chimney Rock, CO	1	Seed	2025	9/17/2002

Table 2.3. Location and habitat of sites visited in 2003.

Humulus	Habitat	Location	Elevation (ft)	County	State
Yes	closed riparian	Oak Cr. Canyon	1713	Coconino	AZ
Yes	closed riparian	Frye Canyon	1965	Graham	AZ
Yes	dry rocks	Lookout Canyon	2174	Coconino	AZ
Yes	dry rocks	Bear Trap Canyon	2303	Socorro	NM
Yes	dry rocks	Carlton Canyon	2646	Lincoln	NM
Yes	dry rocks	Pitchfork Canyon	2697	Graham	AZ
Yes	dry rocks	Windy Point	2798	Lincoln	NM
Yes	open riparian	Macks Crossing	1916	Coconino	AZ
Yes	riparian rocks	Walnut Cr.	2080	Coconino	AZ
Yes	willow	Mt. Lemmon	2306	Pima	AZ
Yes	willow	McNary	2370	Apache	AZ
Yes	willow	Gilita Cr.	2404	Catron	NM
Yes	willow	Alpine	2441	Apache	AZ
No	closed riparian	Summers Spring	1098	Yavapai	AZ
No	closed riparian	Cima Cr.	2196	Cochise	AZ
No	open riparian	Arrastre Cr	1433	Yavapai	AZ
No	open riparian	Animas Cr.	1525	Sierra	NM
No	open riparian	Lower Plaza	1830	Catron	NM
No	open riparian	Show Low Lake	1983	Navajo	AZ
No	open riparian	Tularosa Mtns.	2440	Catron	NM
No	open riparian	Karr Canyon	2592	Otero	NM
No	willow	Blue River	1921	Greenlee	AZ
No	willow	Eagle Cr.	2196	Lincoln	NM

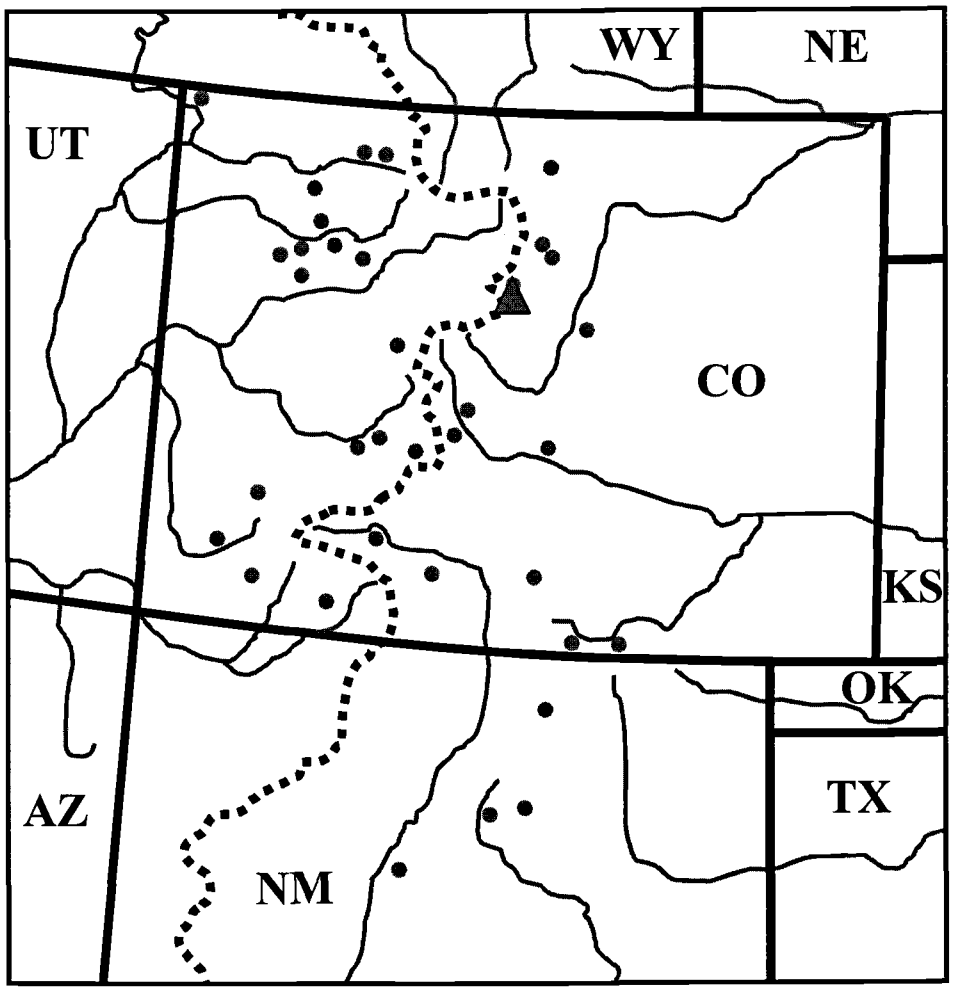

Fig. 2.1. River drainage systems for the region sampled in 2002. The dotted line represents the continental divide. The dots represent collection locations. The triangle represents Silver Plume, the only locality where powdery mildew was observed.

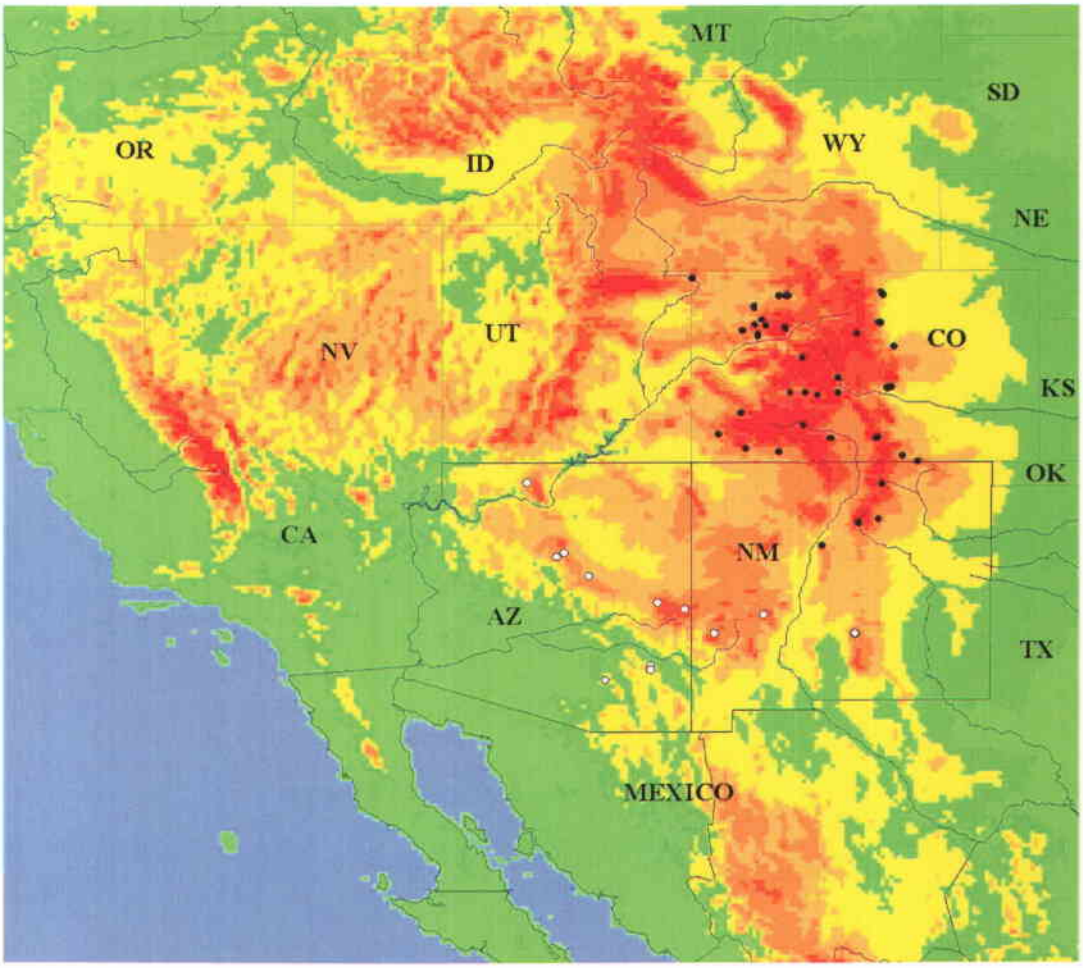

Fig. 2.2. Hop collection sites: 2002 (black dots) and 2003 (white dots).

Conclusions and Further Exploration

Some of the natural range of *H. l.* var. *neomexicanus* in the United States has been successfully sampled. Further collecting from northern Arizona, northwest New Mexico, Nevada, and Utah would complete the survey of this botanical variety. Historic populations along the southern distributional range of *H. lupulus* may become extinct in the future. The genetic diversity within *H. l.* var. *pubescens* is also threatened from both human development and introgression from the naturalized *H. l.* var. *lupulus*. *H. l.* var. *neomexicanus* is poorly represented in *ex situ* collections (Hummer, 2005), so further exploration is recommended. Loss of habitat and introgression are considered to be significant threats to *in situ* conservation efforts in widespread species complexes (Millar and Libby, 1991).

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Chapter 3

Screening of Wild Hop Germplasm for Resistance to Powdery Mildew (*Podosphaera macularis*) Braun & Takamatsu

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Introduction

In 1997, the appearance of hop powdery mildew, caused by *Podosphaera macularis* Braun & Takamatsu (formerly *Sphaerotheca macularis* (Wallr.:Fr.) Lind syn. *S. humuli* (DC.)Burrill), in the Pacific Northwestern United States, threatened commercial hop (*Humulus lupulus* L.) yards due to the lack of resistance incorporated into the current commercial varieties. Approximately 800 of 12,000 hectares in production were destroyed in 1997, due to hop powdery mildew (Ocamb *et al.*, 1999). More than 1,200 hectares of highly susceptible hop varieties were replaced, resulting in an estimated loss of $9.5 million in combined production and establishment costs (Turechek *et al.*, 2001). In 1998, hop powdery mildew was discovered in Idaho and the Willamette Valley of Oregon. Powdery mildew and its control is estimated to have cost Pacific Northwest hop growers over $30 million in 1999 and 2000, or about 15% of their total crop revenue (Turechek *et al.*, 2001). Management was accomplished through 10 to 13 fungicide applications at a cost of approximately $1000/hectare.

The severity of the disease prompted American breeders and brewers to develop resistant hop cultivars. Therfore, hop collecting expeditions sought the discovery of potentially mildew resistant germplasm. From 1999 to 2003, more than 100 seedlots of open-pollinated, wild *H. lupulus* varieties were collected from North Dakota, Missouri, Colorado, New Mexico and Arizona and from Manitoba and Saskatchewan, Canada (Hampton, *et al.*, 2001; Hummer, 2005). In addition, three seedlots of European *H. lupulus* var. *lupulus* were collected from Kazakhstan in 1993 and 2000.

One objective of this project was to evaluate these wild collected plant materials for resistance to powdery mildew. A total of 2,108 native (1563 North American and 107 Kazakhstani) hop seedlings were germinated from 54 seedlots. From 2001 to 2004, these seedlings were evaluated for powdery mildew resistance after artificial inoculation with an Oregon population of *P. macularis*.

In the screening of the wild *Humulus* seedlings, many levels of resistance to *P. macularis* were observed. As was noticed by Mahaffee, *et al.* (2003), the degree of resistance appeared to be dependent upon ambient greenhouse temperatures. Hop genotypes with no to moderate infection at greenhouse temperatures >30°C, became more susceptible to *P. macularis* when grown at temperatures <30°C. The second objective was thus to study the impact of pre-inoculation temperatures on the susceptibility of the three hop genotypes to *P. macularis*.

Materials and Methods

Screening of Wild Hop Germplasm

Seed used in these studies was obtained from the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), National Clonal Germplasm Repository (NCGR), Corvallis, Oregon. They represented populations of native North American hops: *H. l.* var. *lupuloides* from North Dakota and Manitoba and Saskatchewan, Canada, and *H. l.* var. *pubescens* from Missouri. The seeds were collected in 1999, 2000 and 2001, by Richard Hampton and others, in plant collecting expeditions sponsored by the Hop Research Council (Hampton *et al.*, 2001; Hummer, 2005). Seeds of *H. l.* var. *lupulus* were collected from Kazakhstan in 1993 by P.

Forsline and others, and in 2000 by R. Hannan. Plant exploration trips by J. Oliphant and J. Smith to Colorado, New Mexico and Arizona in 2002 and 2003 (Chapter 2) acquired representative populations of *H. l.* var. *neomexicanus* (GRIN, 2005). Locality information for each seedlot screened for powdery mildew resistance is given (Appendix 3.2).

Germination of these seedlots began in March 2001. Conditions for seed germination included a 5 minute soak in a 10% bleach solution followed by a 10 minute rinse with distilled water. Depending on availability, 10, 25, 50 or 100 seeds from each lot were placed on moist, autoclaved sand in clear, plastic germination boxes. Germination boxes were held in the dark at 4°C for 6 weeks. After this prechilling period, the boxes were moved to a germination chamber where they received 14 hours of light at 26°C followed by 10 hours of dark at 10°C. As the radicles emerged, the seeds were removed and placed in cell packs of potting soil to continue germination under greenhouse conditions.

As the seedlings reached adequate size (approximately 15 cm in length), they were artificially inoculated with powdery mildew. A field population of *P. macularis*, consisting of several single-conidial chain isolates collected from Oregon hop yards, was used as inoculum. The isolates were maintained in a growth chamber through successive transfers on potted *H. l.* 'Symphony' plants grown at 13°C with a 15 h photoperiod.

Inoculum was prepared by collecting infected hop leaves from the growth chamber and washing the conidia from the leaves with a 0.005% (vol/vol) solution of Tween 20 and ultra-pure water (18 mΩ). Using a hand-held atomizer (Nalge Nunc

International, Rochester, NY) plants were inoculated with a spore suspension of 20,000 conidia/ml until just before runoff. Leaves were air-dried within 1 h of starting the preparation of the spore suspension to prevent lysis of the conidia. Subsequent inoculations consisted of periodically mechanically shaking heavily infected leaves or whole plants over plants not showing infection to aid in the spread of spores.

A total of 2,108 native (1563 North American and 107 Kazakhstani) hop seedlings were evaluated for resistance to powdery mildew: 657 were evaluated in 2001, 980 were evaluated in 2002, 438 were evaluated in 2003, and 33 were evaluated in 2004. Seedlings were categorized into 4 groups, depending on the level of infection: 1 = no infection; 2 = tolerance, a hypersensitive response (HR), or only a few small colonies with no active sporulation; 3 = moderate infection or medium sized colonies with moderate to high sporulation (reduced susceptibility); 4 = high infection or many large colonies with high sporulation (susceptible). Plants in categories 1 or 2 were repotted into 3-liter pots and re-inoculated at least 5 times.

Thirty-two genotypes from categories 1 or 2 were selected for further evaluation under field conditions. The selections consisted of 29 native North American genotypes (27 *H. l.* var. *lupuloides* and 2 *H. l.* var. *pubescens*) and 3 native Kazakhstani (*H. l.* var. *lupulus*) genotypes. The selections were planted in the USDA-ARS hop yard, Corvallis, Oregon, in June 2003. Due to space restrictions, only 18 of the 32 genotypes were replicated, for a total of 50 plants in the field evaluation. Hop plants were slow to establish in 2003. Field evaluations were taken in summer 2004. Since no natural mildew infections were observed, plants were artificially inoculated in August 2004. Inoculum was prepared in the field as above without quantifying

spore concentration prior to inoculation. Leaves and cones were inoculated using a hand-held atomizer (Nalge Nunc International, Rochester, NY). After plants were inoculated, the spore suspension was calculated as having 15, 250 conidia/ml. Plants were evaluated for leaf infections beginning one week after inoculation. When possible, 100 cones per plant were harvested and evaluated for mildew resistance using a dissecting microscope. Each cone was scored as + or – indicating the presence or absence of hyphae.

Impact of Pre-inoculation Temperature on Susceptibility

The genotypes selected for this study were chosen because of their range of susceptibility to *P. macularis*. Clonal plants of *H. lupulus* 'Nugget' (resistant), 'Symphony' (susceptible) and a wild Kazakhstani selection, CHUM 1025.007, (hypersensitive/tolerant) were produced from softwood cuttings, planted in 5-cm pots with Sunshine Mix (SunGro Horticulture, Bellevue, WA) and grown under greenhouse conditions with at least a 15 h photoperiod. Plants were fertilized with Osmocote (Scotts-Sierra Horticultural Products Co., Marysville, OH) slow-release (14-14-14), watered as needed, and supplied with Champion 17-17-17 fertilizer with micronutrients (McConkey's, Portland, OR) at each watering. Plants were maintained powdery mildew free by vaporizing sulfur in the greenhouse each night for 4 h.

Three one- to two-month old, potted plants of each of three genotypes were grown for 10 days in growth chambers at 29, 32, and 35°C. To prevent contamination from air-borne conidia, plants were grown inside plastic canisters with a cloth lid to allow for air exchange. The canisters were set in trays filled with water to prevent

conidial contamination from below and to keep the relative humidity high, which prevented the leaves from becoming brittle at the high temperatures. Temperature and relative humidity in the growth chambers were monitored with a HOBO pro Series RH/Temp data logger (Onset Computer Corp., Bourne, MA).

Using a hand-held atomizer, plants were inoculated as above, air dried, then placed into a growth chamber at 18°C (the optimal temperature for infection, growth, and sporulation of *P. macularis*) with a 15 h photoperiod. Non-inoculated plants were placed in the chamber as controls to determine ambient levels of infection.

After 10 days at 18°C, the infection frequency was determined for the adaxial surfaces of the four most apical, fully-expanded leaves at the time of inoculation. The infection frequency was determined by dividing the number of lesions per leaf by the leaf area (cm^2). The infection frequency was then averaged across all leaves for each plant. Leaf area was determined by using the average of two measurements obtained with a Li-Cor LI-3000 leaf area meter (Li-Cor Inc., Lincoln, NE). Observations of infection on leaf surfaces were made using a light microscope (Carl Zeiss, West Germany).

The experiment was a 3 x 3 factorial, arranged in a split-plot design with sub-sampling. Temperature served as the whole-plot factor and genotype served as the sub-plot factor. The experimental unit was the growth chamber. The response was the infection frequency: the total number of lesions on 12 leaves (4 leaves from each of 3 plants) divided by the total leaf area of the 12 leaves. Temperatures were randomly assigned to growth chambers for each replication. The experiment was replicated three times with replication in time serving as the blocking factor. The

assumptions of normality and homogeneity of variance were checked in SAS (SAS Institute, Cary, NC). Results (Table 3.7) were analyzed on the non-transformed data for 'Symphony' and KAZ 1025.007 using a three-way analysis of variance (Ramsey and Schafer, 2002). The three way interaction (*temperature* x *genotype* x *block*) was used as the experimental error. The 'Nugget' response was not analyzed because the infection frequency was 0 throughout the testing.

This experiment was designed to examine the effect of temperature on the specific genotypes, while eliminating the effect of temperature on *P. macularis.* This was accomplished by subjecting the plants to temperature treatments prior to inoculation. The fungus was then introduced into a temperature treatment optimum for its growth (18°C).

Results and Discussion

Screening of Wild Hop Germplasm

In 2001, 657 hop seedlings from 27 North American seedlots were screened for powdery mildew resistance. When average daily maximum greenhouse temperatures were >30°C, 66 seedlings appeared to have some level of resistance, and grouped into categories 1 and 2 with 6 seedlings having no visible infection (Table 3.1). Of these 66 seedlings, 46 (or 70%) came from 2 seedlots of *H. l.* var. *lupuloides*: Souris-E2 from Manitoba and Logan-N from North Dakota.

In March of 2002, when daily maximum greenhouse temperatures averaged <30°C, only 22 seedlings appeared to have some resistance with only one seedling having no visible infections (Table 3.2). This indicated that the observed resistance

was related to the plants response to temperature. Of the 22 seedlings showing resistance at cooler temperatures, 15 (or 68%) came from 2 seedlots of *H. l.* var. *lupuloides*: Souris-E2 from Manitoba and Logan-N from North Dakota.

Additional seedlings were germinated in the spring of 2002 (Tables 3.3 and 3.4). These included 9 seedlots that had produced resistant/tolerant genotypes in 2001, as well as 7 previously unscreened seedlots from North Dakota (*H. l.* var. *lupuloides*) and Kazakhstan (*H. l.* var. *lupulus*). A total of 980 seedlings were inoculated with powdery mildew, grown at greenhouse temperatures >30°C, and evaluated for powdery mildew resistance (Table 3.3).

Of the 980 seedlings evaluated (Table 3.3), 151 appeared to have some resistance and grouped into categories 1 and 2, with 68 having no visible infection. Thus, resistance was observed in 15.4% of the seedlings screened. Of these 151 seedlings showing resistance, 92 (61%) were from 3 seedlots of *H. l.* var. *lupuloides*: 22 (15%) from Souris-E2 (Manitoba), 26 (17%) from Burlington N2 #2 (North Dakota), and 44 (29%) from Foxholm-N (North Dakota). Shortly after these evaluations were made, seedlings in categories 1 and 2 were moved to a cooler (average daily maximum greenhouse temperatures <30°C) greenhouse, located at ARS, Horticultural Crops Research Laboratory, Corvallis, Oregon, and inoculated and evaluated for resistance at cooler temperatures (Table 3.4).

Table 3.1. Powdery mildew infection categories[1] of *Humulus lupulus* var. *lupuloides* and var. *pubescens* seedlots (collected in 1999) after inoculation in summer 2001. Evaluations were made on 10/02/2001. Average maximum daily greenhouse temperatures were greater than 30°C.

Seedlot Identity	Collection Locality	Botanical Variety	Infection Category				Total
			1	2	3	4	
Souris-E2	Manitoba	*lupuloides*	3	23	8	15	49
Logan-N	North Dakota	*lupuloides*	1	19	5	13	38
Burlington-N	North Dakota	*lupuloides*	0	3	8	40	51
Burlington-N#2	North Dakota	*lupuloides*	0	1	4	30	35
Minot-E	North Dakota	*lupuloides*	0	0	0	9	9
White Earth-S	North Dakota	*lupuloides*	0	0	2	14	16
White Earth-S2	North Dakota	*lupuloides*	0	0	3	24	27
Little Knife-E	North Dakota	*lupuloides*	0	0	0	6	6
Oxbow-S	Saskatchewan	*lupuloides*	1	2	1	14	18
Indian Head-N	Saskatchewan	*lupuloides*	0	0	0	11	11
Bridge 2S	Saskatchewan	*lupuloides*	0	0	2	13	15
2 Qu'Appelle	Saskatchewan	*lupuloides*	0	1	0	31	32
3 Qu'Appelle	Saskatchewan	*lupuloides*	0	0	0	21	21
Grenfell-N	Saskatchewan	*lupuloides*	0	0	1	18	19
Melville-S	Saskatchewan	*lupuloides*	0	0	1	9	10
4 Qu'Appelle	Saskatchewan	*lupuloides*	0	0	0	1	1
Lisbon-NW	North Dakota	*lupuloides*	0	1	0	1	2
Fort Ransom	North Dakota	*lupuloides*	0	0	1	21	22
Rulo-E #1	Missouri	*pubescens*	0	6	1	73	80
Rulo-E #2	Missouri	*pubescens*	0	2	2	58	62
Rulo-E #3	Missouri	*pubescens*	0	0	3	38	41
Rulo-E #4	Missouri	*pubescens*	0	1	3	36	40
Rulo-E #5,6	Missouri	*pubescens*	1	0	0	4	5
Rulo-E #7	Missouri	*pubescens*	0	0	0	3	3
Rulo-E #8	Missouri	*pubescens*	0	0	0	1	1
Rulo-E #9	Missouri	*pubescens*	0	1	1	40	42
Rulo-E2	Missouri	*pubescens*	0	0	0	1	1
		Totals:	**6**	**60**	**46**	**545**	**657**
		%	**1**	**9**	**7**	**83**	

[1] 1 = no infection; 2 = tolerance, hypersensitive response (HR), or only a few small colonies with no active sporulation; 3 = moderate infection or medium sized colonies with moderate to high sporulation (reduced susceptibility); 4 = high infection or many large colonies with high sporulation (susceptible).

Table 3.2. Powdery mildew infection categories[1] of *Humulus lupulus* var. *lupuloides* and var. *pubescens* seedlots, collected in 1999. Evaluations were made on 3/6/2002. Average maximum daily greenhouse temperatures were less than 30°C.

Seedlot Identity	Collection Locality	Botanical Variety	Infection Category				Total
			1	2	3	4	
Souris-E2	Manitoba	*lupuloides*	0	11	17	21	49
Logan-N	North Dakota	*lupuloides*	0	4	20	14	38
Burlington-N	North Dakota	*lupuloides*	0	2	9	40	51
Burlington-N#2	North Dakota	*lupuloides*	0	0	1	34	35
Minot-E	North Dakota	*lupuloides*	0	0	0	9	9
White Earth-S	North Dakota	*lupuloides*	0	1	1	14	16
White Earth-S2	North Dakota	*lupuloides*	0	0	3	24	27
Little Knife-E	North Dakota	*lupuloides*	0	0	0	6	6
Oxbow-S	Saskatchewan	*lupuloides*	0	0	4	14	18
Indian Head-N	Saskatchewan	*lupuloides*	0	0	0	11	11
Bridge 2S	Saskatchewan	*lupuloides*	0	0	2	13	15
2 Qu'Appelle	Saskatchewan	*lupuloides*	0	0	1	31	32
3 Qu'Appelle	Saskatchewan	*lupuloides*	0	0	0	21	21
Grenfell-N	Saskatchewan	*lupuloides*	1	0	0	18	19
Melville-S	Saskatchewan	*lupuloides*	0	0	1	9	10
4 Qu'Appelle	Saskatchewan	*lupuloides*	0	0	0	1	1
Lisbon-NW	North Dakota	*lupuloides*	0	0	1	1	2
Fort Ransom	North Dakota	*lupuloides*	0	0	1	21	22
Rulo-E #1	Missouri	*pubescens*	0	2	5	73	80
Rulo-E #2	Missouri	*pubescens*	0	0	4	58	62
Rulo-E #3	Missouri	*pubescens*	0	0	3	38	41
Rulo-E #4	Missouri	*pubescens*	0	0	4	36	40
Rulo-E #5,6	Missouri	*pubescens*	0	1	0	4	5
Rulo-E #7	Missouri	*pubescens*	0	0	0	3	3
Rulo-E #8	Missouri	*pubescens*	0	0	0	1	1
Rulo-E #9	Missouri	*pubescens*	0	0	2	40	42
Rulo-E2	Missouri	*pubescens*	0	0	0	1	1
		Totals:	**1**	**21**	**79**	**556**	**657**
		%	**0.2**	**3.2**	**12**	**84.6**	

[1] 1 = no infection; 2 = tolerance, hypersensitive response (HR), or only a few small colonies with no active sporulation; 3 = moderate infection or medium sized colonies with moderate to high sporulation (reduced susceptibility); 4 = high infection or many large colonies with high sporulation (susceptible).

Table 3.3. Powdery mildew infection categories[1] of *Humulus lupulus* var. *lupuloides*, var. *pubescens*, and var. *lupulus* seedlots collected in 1993, 1999, 2000 and 2001. Evaluations were made on 8/26/2002. Average maximum daily greenhouse temperatures were greater than 30°C.

Seedlot Identity	Collection Locality	Botanical Variety	Infection Category				Total
			1	2	3	4	
Foxholm-N	North Dakota	*lupuloides*	3	41	97	85	226
MP73-W (#1)	North Dakota	*lupuloides*	2	4	10	12	28
MP73-W (#2)	North Dakota	*lupuloides*	0	1	11	58	70
Carpio-S	North Dakota	*lupuloides*	1	1	11	9	22
Souris E-2	Manitoba	*lupuloides*	20	2	21	5	48
Logan-N	North Dakota	*lupuloides*	8	6	3	4	21
Burlington N2 #2	North Dakota	*lupuloides*	13	13	102	103	231
Oxbow-S	Saskatchewan	*lupuloides*	8	1	6	6	21
Indian Head-N	Saskatchewan	*lupuloides*	0	0	3	19	22
Bridge 2S	Saskatchewan	*lupuloides*	7	1	17	15	40
3 Qu'Appelle	Saskatchewan	*lupuloides*	1	3	12	29	45
Rulo-E #1	Missouri	*pubescens*	0	2	6	6	14
Rulo-E #9	Missouri	*pubescens*	3	7	36	72	118
KAZ 804	Kazakhstan	*lupulus*	0	0	0	47	47
KAZ1024	Kazakhstan	*lupulus*	0	0	0	7	7
KAZ1025	Kazakhstan	*lupulus*	2	1	0	17	20
		Totals:	**68**	**83**	**335**	**494**	**980**
		%	**7**	**9**	**34**	**50**	

[1] 1 = no infection; 2 = tolerance, hypersensitive response (HR), or only a few small colonies with no active sporulation; 3 = moderate infection or medium sized colonies with moderate to high sporulation (reduced susceptibility); 4 = high infection or many large colonies with high sporulation (susceptible).

Table 3.4. Powdery mildew infection categories[1] of *Humulus lupulus* var. *lupuloides*, var. *pubescens*, and var. *lupulus* seedlots collected in 1993, 1999, 2000 and 2001. Evaluations were made on 10/10/2002. Average daily greenhouse temperatures were less than 30°C.

Seedlot Identity	Collection Locality	Botanical Variety	Infection Category				Total
			1	2	3	4	
Foxholm –N	North Dakota	*lupuloides*	0	1	18	207	226
MP73-W (#1)	North Dakota	*lupuloides*	0	1	2	25	28
MP73-W (#2)	North Dakota	*lupuloides*	0	0	0	70	70
Carpio-S	North Dakota	*lupuloides*	0	0	0	22	22
Souris E-2	Manitoba	*lupuloides*	0	3	1	44	48
Logan-N	North Dakota	*lupuloides*	0	0	6	15	21
Burlington N2 #2	North Dakota	*lupuloides*	0	13	2	216	231
Oxbow-S	Saskatchewan	*lupuloides*	0	1	0	20	21
Indian Head-N	Saskatchewan	*lupuloides*	0	0	0	22	22
Bridge 2S	Saskatchewan	*lupuloides*	0	0	0	40	40
3 Qu'Appelle	Saskatchewan	*lupuloides*	1	0	0	44	45
Rulo-E #1	Missouri	*pubescens*	0	0	1	13	14
Rulo-E #9	Missouri	*pubescens*	0	0	2	116	118
Kazakhstan 804	Kazakhstan	*lupulus*	0	0	0	47	47
Kazakhstan 1024	Kazakhstan	*lupulus*	0	0	0	7	7
Kazakhstan 1025	Kazakhstan	*lupulus*	3	0	0	17	20
		Totals:	**4**	**19**	**32**	**925**	**980**
		%	**0.4**	**1.9**	**3.3**	**94.4**	

[1] 1 = no infection; 2 = tolerance, hypersensitive response (HR), or only a few small colonies with no active sporulation; 3 = moderate infection or medium sized colonies with moderate to high sporulation (reduced susceptibility); 4 = high infection or many large colonies with high sporulation (susceptible).

As hypothesized, the total number of resistant seedlings decreased at cooler temperatures, with only 23 seedlings being grouped into categories 1 and 2, with only 4 seedlings having no visible infection. Of these 23 seedlings, 13 (or 57%) were from Burlington-N2 #2, North Dakota, 3 (or 13%) were from Souris-E2, Manitoba, and 3 (or 13%) were from Kazakhstan, KAZ 1025.

In 2003, a total of 438 *H. l.* var. *neomexicanus* seedlings from 20 lots (collected from Colorado in 2002) were evaluated for powdery mildew resistance (Table 3.5). Of these seedlings, 16 (or 3.7%) were grouped into category 2. Of these 16 seedlings, 10 (or 62.5%) came from two lots collected in Redstone Creek and Silver Plume, Colorado.

In 2004, 33 seedlings from Kazakhsani seedlot 1025 (in which resistance was found in 2002) were evaluated. Of these, only 1 seedling showed resistance. The resistance observed in this seedling appeared to be morphologically similar to the 3 Kazakhstani seedlings from the 2002 evaluation (Tables 3.3 and 3.4), where at 18°C, necrotic lesions formed at the center of colonies and enlarged over time.

Darby *et al.* (1989) looked at the association between powdery mildew infections on seedlings and adult plants using a contingency χ^2 test. They reported significant positive associations between the severity of infection on seedling leaves and cones and between the severity of infection on mature leaves and cones. A significant positive association was also observed in males between the severity of infection of seedlings and that on mature plants. Thus, illustrating the efficiency of selecting for disease resistance at the seedling stage as opposed to waiting to evaluate cone infection.

Table 3.5. Powdery mildew infection categories[1] of *Humulus lupulus* var. *neomexicanus* seedlots (collected from Colorado in 2002). Seedlings were grown at 18-20C.

Seedlot Identity	Collection Locality	Botanical Variety	Infection Category				Total
			1	2	3	4	
Coal Creek	Colorado	*neomexicanus*	0	2	6	18	26
Mesa Trailhead (1)	Colorado	*neomexicanus*	0	0	4	21	25
Rattlesnake Gulch (2)	Colorado	*neomexicanus*	0	0	6	26	32
Eldorado Canyon S.P. (3)	Colorado	*neomexicanus*	0	1	4	25	30
Eldorado Springs (4)	Colorado	*neomexicanus*	0	2	10	22	34
Redstone Creek 1	Colorado	*neomexicanus*	0	5	12	16	33
Redstone Creek 2	Colorado	*neomexicanus*	0	0	6	9	15
Hayden 1	Colorado	*neomexicanus*	0	1	10	23	34
Axial 3	Colorado	*neomexicanus*	0	0	6	20	26
Sweetwater Lake 1	Colorado	*neomexicanus*	0	0	0	9	9
Sweetwater Lake 2	Colorado	*neomexicanus*	0	0	0	31	31
Sweetwater Lake 3	Colorado	*neomexicanus*	0	0	0	28	28
Silver Plume	Colorado	*neomexicanus*	0	5	8	10	23
Aspen	Colorado	*neomexicanus*	0	0	0	2	2
Willow Creek 1	Colorado	*neomexicanus*	0	0	0	2	2
Cochetopa Canyon	Colorado	*neomexicanus*	0	0	3	13	16
Tomichi Creek	Colorado	*neomexicanus*	0	0	0	18	18
Sangre de Cristo 2	Colorado	*neomexicanus*	0	0	1	1	2
Chimney Rock	Colorado	*neomexicanus*	0	0	0	28	28
Leopard Creek 2	Colorado	*neomexicanus*	0	0	0	24	24
		Totals:	**0**	**16**	**76**	**346**	**438**
		%	**0**	**4**	**17**	**79**	

[1] 1 = no infection; 2 = tolerance, hypersensitive response (HR), or only a few small colonies with no active sporulation; 3 = moderate infection or medium sized colonies with moderate to high sporulation (reduced susceptibility); 4 = high infection or many large colonies with high sporulation (susceptible).

Based on the 2001-2003 greenhouse screenings, 32 native hop genotypes were selected for their resistance to powdery mildew. The selected genotypes consisted of 10 females, 16 males, 4 monoecious plants and 2 that did not flower (Appendix 3.1). The 4 monoecious plants were collected from North Dakota and 3 showed signs of monoecy for the first time in 2004.

Natural powdery mildew infections were not observed on any of the plants before artificial inoculation in August 2004, despite significant levels of hop mildew in adjacent yards. One week after inoculation, small sporulating colonies were observed on the leaves of only one genotype, a female *H. l.* var. *pubescens* from Rulo-E, Missouri (1019#1-45). Tiny necrotic lesions were evident on the leaves of the three *H. l.* var. *lupulus* from Kazakhstan (1025-01, -10, -18). All *H. l.* var. *lupuloides* and one *H. l.* var. *pubescens* (1019#5,6-05) exhibited foliar resistance to powdery mildew under field conditions. Thus, resistance observed in greenhouse screenings, was consistent in the field.

The percentage of cones infected per plant ranged from 0 to 100% (Appendix 3.1). In most cases, cone infections were limited and only visible under a dissecting scope. On some cones, tiny, brown flecks could be seen with the unaided eye, indicative of the presence of mildew. Hyphal mass was minimal on these cones and only a few conidiophores were present per colony. This was in stark contrast to infected cones from susceptible genotypes, such as 1019#1-45, in which sporulating lesions could be seen with the unaided eye, and cone distortion occurred.

Genotypes with the smallest percentage of cone infections came from seedlots of *H. l.* var. *lupuloides* collected near Souris, Manitoba, and Logan and Burlington,

North Dakota, as well as *H. l.* var. *lupulus* collected near Emba, Kazakhstan. The female 1000-24, from Souris, Manitoba had 0% cone infections. Two females from Logan, ND, (1001-02 and 1001-03), were each replicated twice and had 3.5% (4.95 SD) and 9% (8.49 SD) mean cone infections, respectively. Two monoecious plants had little to no cone infections: plant 1001-24 from Logan, ND had 0% cone infections while 1002-36 from Burlington, ND, had 2% (2.83 SD) mean cone infections (two clonal replicates). One of the three plants from Kazakhstan had cones with 0% cone infection, although the cones were extremely small, ranging from 1 to 1½ cm in length.

This study has found resistance to powdery mildew in hops from the northern plains region of the United States and Canada, and from Kazakhstan. There was variability in the degree of resistance both within and between the seedlots evaluated. The frequency of resistance for each of the four botanical varieties was calculated (Table 3.6). The highest frequency was found in *H. l.* var. *lupuloides* (7.7%), followed by 3.7% in *H. l.* var. *lupulus* and 1.2% in *H. l.* var. *pubescens*. High levels of resistance were not observed in the *H. l.* var. *neomexicanus* seedlots. The North American localities where resistance was observed (Fig. 3.1) did not correlate with latitude, however, most of the resistant types were found in the Manitoba-Saskatchewan-North Dakota region.

Table 3.6. Frequency of powdery mildew resistance in populations of four botanical varieties of *H. lupulus* from North America and Kazakhstan.

Botanical Variety	# Resistant	Total # Screened	Frequency of Resistance
lupuloides	89	1156	7.7
pubescens	5	407	1.2
lupulus	4	107	3.7
neomexicanus	0	438	0

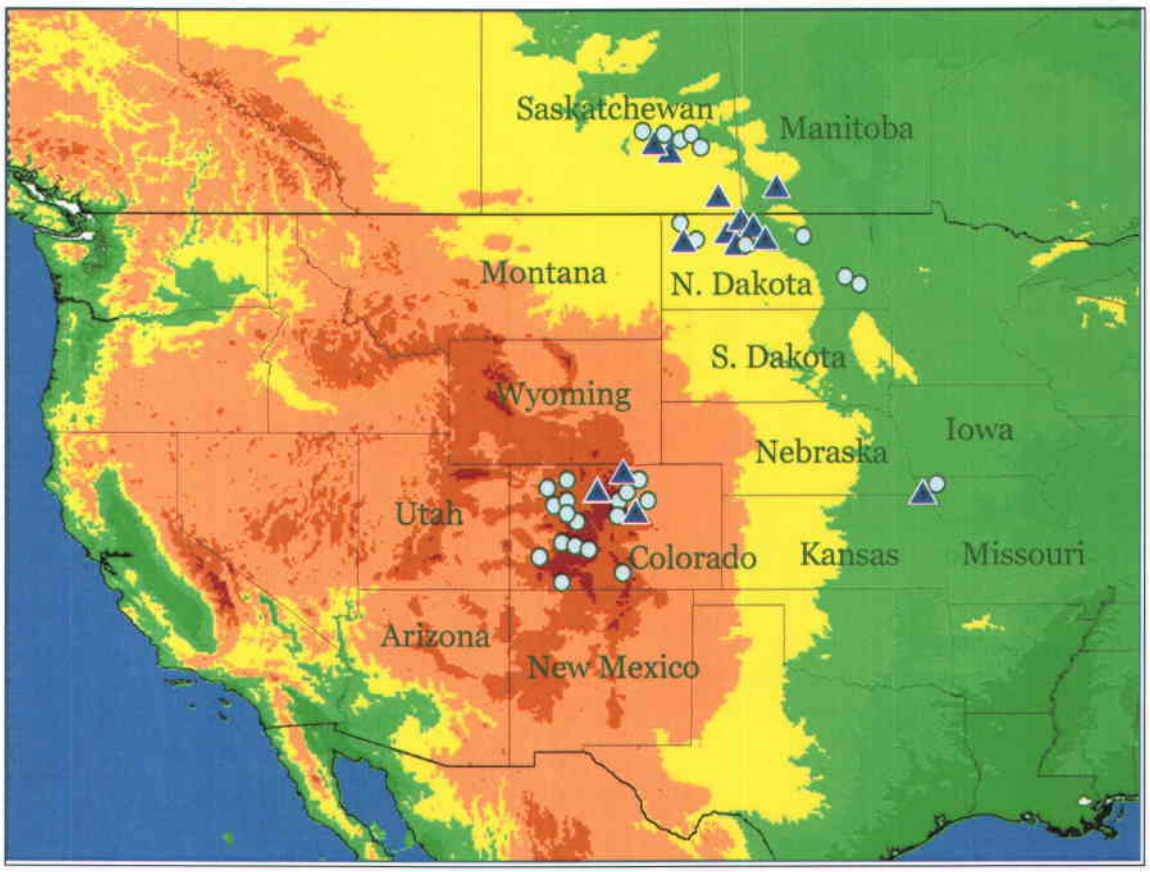

Fig. 3.1. *H. l.* var. *lupuloides*, var. *pubescens*, and var. *neomexicanus* seedlots screened for powdery mildew resistance. Blue triangles indicate seedlots where resistance was observed.

Selected *H. l.* var. *lupuloides* from North America and *H. l.* var. *lupulus* from Kazakhstan may have the polygenic form of resistance discussed by Darby (1998). In this type of resistance there is decreased lesion size and reduced sporulation of mildew colonies. The genes involved in this form of resistance should prove useful to breeders because the selection pressure for new pathotypes of *P. macularis* to develop is reduced. The seven known resistance genes in cultivated hops have each been overcome by more virulent strains of mildew. A polygenic form of resistance would thus be useful to growers, in that the number of fungicide applications necessary for control could be considerably reduced.

Native hops have morphological traits that could prove useful to breeding programs. Hampton (2000) describes *H. l.* var. *pubescens* as "certainly the premier population of the lower Missouri River region, including plants that arguably produce the highest ratio of cones per unit-plant-mass in North America, with some of the most favorable cone/plant type attributes." Hampton *et al.* (2001) suggested that *H. l.* var. *pubescens* might have defenses adapted to local insect species due to its exceptional pubescence. The most notable discovery made in a numerical taxonomic analysis of morphological characters by Small (1980), was the remoteness of the relationship of 79 European, Japanese and North American hop cultivars to *H. l.* var. *pubescens*. The geographical and morphological distinctiveness of *H. l.* var. *pubescens* suggests that this wild variety could be useful for improving hop cultivars (Small, 1980).

Genotypes selected in this study have been deposited at the USDA, ARS, National Clonal Germplasm Repository, Corvallis, Oregon. They are available to researchers and breeders upon request.

Impact of Pre-inoculation Temperature on Susceptibility

Pre-inoculation temperature had a significant effect ($p < 0.05$) on infection frequency, while the effect of genotype was highly significant ($p < 0.01$), (Table 3.7). There was no effect of a temperature x genotype interaction ($p > 0.05$).

When exposed to high temperatures prior to inoculation, 'Symphony' exhibited a reduced susceptibility to *P. macularis* (Fig. 3.2), confirming the findings of Mahaffee *et al.* (2003). The pre-existing partial resistance of the Kazakhstani genotype was also increased by exposure to high temperatures prior to inoculation (Fig. 3.2). No infection was found to occur on 'Nugget' at any temperature treatment (Fig. 3.2). These results imply that susceptible *Humulus* genotypes exhibit temperature dependent resistance.

The majority of spores germinated on each genotype within 15 to 24 hours of inoculation. On 'Symphony', at 29°C, spore germination occurred normally with 4 to 5 germ tubes developing within 2 to 3 days. At 29°C, germ tube growth on the Kazakhstani selection was similar but delayed. After 3 days, epidermal leaf cells began to collapse, leading to hyphal death. On 'Nugget' at any temperature treatment, conidia germinated but did not develop past the initial germ tube before collapsing; no further fungal development or sporulation occurred. The infection process progressed more slowly on plants grown at higher temperatures prior to inoculation. Temperatures of 32 and 35°C significantly slowed the hyphal growth rate, and delayed sporulation on 'Symphony' and the Kazakhstani selection. On 'Symphony', grown at 35°, conidia had not progressed past the primary germ tube (1 septa) by 72 h. When grown at 18° C prior to inoculation, by 72 h, spores on 'Symphony' had already

produced 4 to 5 germ tubes with primary branching (Chapter 4: Table 4.1, Fig. 4.1). Interestingly, noticeably fewer spores were found on all genotypes given a 35°C pre-inoculation temperature as opposed to those receiving < 32°C temperature treatments. It is possible that spores are less likely to adhere to leaf surfaces grown at 35°C. This could be due to a probable difference in the quality or quantity of cuticular wax.

Table 3.7. Analysis of Variance (ANOVA) results.

	Df	Sum of Sq	Mean Sq	F Value	Pr (F)
Temp	2	7.586571	3.793286	17.0022	< 0.05
Genotype	1	8.191803	8.191803	36.0434	< 0.01
Block	2	0.828152	0.414076		
Temp:Genotype	2	1.509940	0.754970	3.3218	> 0.05
Temp:Block	4	0.892421	0.223105		
Temp:Genotype:Block	6	1.363657	0.227276		
Total	17				

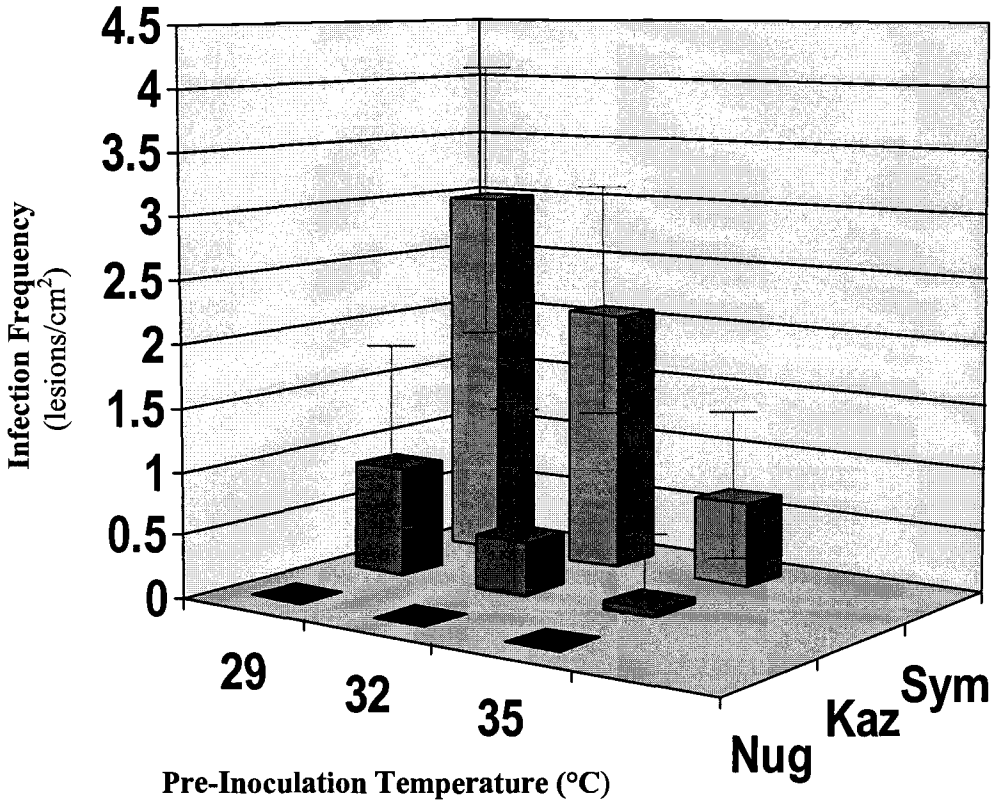

Fig. 3.2. Mean infection frequency of powdery mildew on 'Nugget', Kaz 1025.007, and 'Symphony' after exposure to pre-inoculation temperatures of 29, 32, or 35°C for 10 days. Disease was assessed 10 days after inoculation and incubation at 18°.

The results of this study suggest that the degree of temperature dependent resistance increases with the susceptibility of the hop genotype. Immune genotypes remained consistently uninfected at the test temperature regimes. The resistance of moderately resistant genotypes was increased by exposure to high temperatures, while the degree of resistance in susceptible genotypes was dramatically increased. Munger (1979) observed the influence of temperature on the resistance of cucumber to powdery mildew. In that study, the effect of temperature was more pronounced on cucumbers with intermediate levels of powdery mildew resistance than on susceptible genotypes.

Possibly either a physical modification, such as the thickening of the leaf cuticle, or a more complex biochemical alteration is occurring in moderate to susceptible hop genotypes when exposed to high temperatures (> 29°C). Future studies should determine if the resistance of 'Nugget', and the temperature dependent resistance of 'Symphony', is derived from the relative difficulty of the fungus to penetrate a thicker cuticle. Studies of powdery mildew on other crops have shown that the difference in resistance between genotypes was due to cuticle thickness. Jhooty and McKeen (1965) compared the thickness of the cuticle in two strawberry species. The thickness of the upper cuticle of a leaf of *Fragaria chiloensis* Duchesne (resistant to strawberry powdery mildew caused by *Sphaerotheca macularis* (Wallr. ex Fries) Jaczewski on upper leaf surface) was almost seven times that of the upper cuticle of *Fragaria ovalis* Wils. [syn = *F. virginiana subsp. platypetala* (Rydb.) Stout] (susceptible).

Peries (1962) observed that susceptible and resistant strawberry varieties differed by the ease with which the fungus penetrated the host cuticle and epidermal wall. Surface wax content did not significantly differ among the strawberry varieties, but the amount of cutin acids from resistant varieties was higher than those from susceptible varieties. Peries' studies with cutin acids suggest that fungitoxic materials can be derived from the cutin of strawberry leaves.

Similar observations were observed in rose varieties with resistance to *S. pannosa* (Wallr. ex Fr.) Lev (Mence and Hildebrandt, 1966). A correlation was found between the combined thickness of the cuticle and epidermal cell wall and the degree of resistance. This correlation, however, did not extend among varieties; the degree of resistance was age-related and not primarily dependent on cuticle morphology.

In contrast, no correlation between cuticle thickness and resistance to powdery mildew was observed in the following interactions: apple (*Malus domestica* L.) - *Podosphaera leucotricha* (Ell. & Ev.) Salm. (Cimanowski *et al.*, 1975); lettuce (*Lactuca sativa* L.)-*Erysiphe cichoracearum* (DC. Ex Merat) (Schnathorst, 1959); and *Plox* sp.-*Erysiphe cichoracearum* DC. (Jarosz *et al.*, 1982).

The upper and lower epidermal cells of mature hop leaves show numerous cuticular folds covered with a continuous layer of wax devoid of any sculpturing or crystalloids. Gülz *et al.* (1993) found that removal of this layer with chloroform, ($CHCl_3$), had no effect on the cuticular foldings, demonstrating that these lamellate or undulate folds consist not of wax but of cutin. The epicuticular wax layer may provide a degree of physical defense against the ingress of fungal spores. Gülz *et al.* (1993) suggest that the complex chemical composition of the wax layer may have a

specific role in protection and that a fungistatic function for leaf-wax components could be considered.

The resistance observed in the Kazakhstani genotype (CHUM 1025.007), might be due to fungistatic chemical properties either inherent or produced in response to infection. In this genotype, the pathogen was able to penetrate the host, but growth and subsequent sporulation was hindered. Epidermal cells began to collapse at the center of infection, forming a necrotic lesion that enlarged in circumference over time. The collapse of epidermal cells in advance of hyphal collapse suggests that fungistatic conditions developed in response to infection. The unusual type of resistance exhibited by the Kazakhstani genotype suggests that it may be a candidate for a future germplasm release. Although the genes involved are unknown at this time, this study might inspire research directed at the identification of these resistance genes.

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Chapter 4

Microscopic Observations of the Progression of Powdery Mildew (*Podosphaera macularis* Braun & Takamatsu) on Native North American and European Hop Leaves

Jodi M. Smith, Kim E. Hummer, and Walter F. Mahaffee

Introduction

The 1997 appearance of hop powdery mildew, caused by *Podosphaera macularis* (formerly *Sphaerotheca macularis* (Wallr.:Fr.) Lind syn. *S. humuli* (DC.)Burrill), in the Pacific Northwestern United States was devastating to the hop (*Humulus lupulus* L.) industry. The lack of resistance in commercial hop cultivars led to total crop loss, reductions in yield, and numerous costly fungicide applications. In Washington State, more than 1,200 hectares of highly susceptible hop varieties were replaced in 1997, resulting in an estimated loss of $9.5 million in combined production and establishment costs (Turechek *et al.*, 2001). The United States Department of Agriculture, the hop industry, and the Hop Research Council supported research directed at the discovery of resistant germplasm. Collection trips acquired over 100 seedlots of native *H. l.* var. *lupuloides* Small, *H. l.* var. *pubescens* Small, and *H. l.* var. *neomexicanus* Nelson and Cockerell, from the United States and Canada (Hampton *et al.*, 2001; Hummer, 2005.). Two collection trips to Kazakhstan acquired 3 seedlots of wild *H. l.* var. *lupulus*. This germplasm was screened for resistance to an Oregon population of *P. macularis* under greenhouse and field conditions (Chapter 3). Several genotypes expressed partial resistance in the form of reduced number of lesions, lesion size and sporulation.

Previous microscopic observations of primarily European pathotypes of *P. macularis* have been made on European hop cultivars (Liyanage, 1973, as cited by Royle, 1978; Godwin, 1985; Godwin *et al.*, 1987). The objective of this study was to microscopically observe the development of an Oregon population of *P. macularis* on susceptible, resistant, and partially resistant hop genotypes.

Materials and Methods

Clonal plants of 'Symphony' (susceptible), 'Nugget' (resistant), 'Newport' (resistant), 7 native North American genotypes (partially resistant) [6 *H. l.* var. *lupuloides* (1000.020, 1000.026, 1000.028, 1000.029, 1000.030, 1008.010) and 1 *H. l.* var. *pubescens* (1019.037)], and 3 native Kazakhstani [*H. l.* var. *lupulus* (1025.001, 1025.007, 1025.010)] genotypes (partially resistant) were produced from softwood cuttings, planted in 5-cm square pots with Sunshine Mix (SunGro Horticulture, Bellevue, WA) and grown under greenhouse conditions (18 to 24°C) with at least a 15 h photoperiod. Plants were fertilized with Osmocote slow-release (14-14-14), watered as needed and supplied with Champion 17-17-17 fertilizer with micronutrients (McConkey's, Portland, OR) at each watering. Plants were maintained powdery mildew free by vaporizing sulfur in the greenhouse each night for 4 h.

A field population of *P. macularis*, consisting of several single-conidial chain isolates collected from Oregon hop yards was used as inoculum. The isolates were maintained in a growth chamber through successive transfers on potted 'Symphony' plants grown at 13°C with a 15 h photoperiod. Inoculum was prepared by collecting infected hop leaves from the growth chamber and washing the conidia from the leaves with a 0.005% (vol/vol) solution of Tween 20 and ultra-pure water (18 mΩ). Using a hand-held Nalgene atomizer (Nalge Nunc International, Rochester, NY), plants were inoculated with a spore suspension of 20,000 conidia/ml until just before runoff. Leaves were air-dried within 1 h of preparation of the spore suspension to prevent lysis of the conidia and placed into a growth chamber at 18°C with a 15 h photoperiod.

Progression of the disease was observed on intact plant leaves over time, using light microscopy (Carl Zeiss, West Germany). Three types of observations - stage of fungal growth, host response, and colony area, were made. These measurements were taken from two plants of each genotype. To determine the stage of fungal growth and host response, the 2 fully expanded leaves at the second node from the growing tip, at the time of inoculation, were observed at 7 intervals between 0 and 168 hours after inoculation. Colony area was measured for 2 colonies per leaf, 2 leaves plant. Colony area was determined using a stage micrometer.

Results

The infection progress on the different genotypes is described (Tables 4.1, 4.2 and 4.3, Appendix 4.1). Conidial germination occurred most rapidly on the susceptible cultivar 'Symphony'. The first conidia germinated 7 h after inoculation (Table 4.1) and by 15 h, each of the conidia observed had germinated. Preliminary studies indicated that 'Symphony' had as many as 250 germinated conidia per leaf (data not shown). Primary branching of hyphae was observed at 48 h (Appendix 4.1). Secondary branching and conidiophore initials were apparent by 96 h, and sporulation occurred by 120 h (Appendix 4.1).

Some conidia began germinating on the resistant varieties 'Nugget' and 'Newport' after 15 h, with others germinating by 48 h. Conidia did not develop past the initial germ tube (one septa) on these genotypes.

Conidia began germinating between 15 and 24 h on *H. l.* var. *lupuloides* and *H. l.* var. *pubescens* (Table 4.1). Although germination of some conidia began at 15 h on *H. l.* var. *lupulus*, additional conidia were still germinating at 48 h. Primary branching was observed after 48 h on *H. l.* var. *lupuloides* genotypes, but not until 72 hours on *H. l. pubescens* and *H. l.* var. *lupulus* genotypes (Table 4.1). Secondary branching and conidiophore initials appeared after 96 h on *H. l.* var. *lupuloides*. Although secondary branching was also observed after 96 h on *H. l.* var. *lupulus*, conidiophore initials did not appear until 120 h. Secondary branching did not occur on *H. l.* var. *pubescens* until 120 h, with conidiophore initials appearing after 144 h. Although sporulation was observed in a few colonies on *H. l.* var. *lupuloides* and *H. l.* var. *lupulus* after 120 h, most colonies on these genotypes did not sporulate until 144 h. Sporulation occurred after 168 h on *H. l.* var. *pubescens*.

The host response to the presence of *P. macularis* varied by genotype (Table 4.2). 'Symphony' showed no defense response to penetration by the fungus (Fig. 4.1). Leaf tissues remained green, and the fungus developed normally.

There was no apparent defense response observed on either 'Nugget' or 'Newport'. The germinated conidium collapsed shortly after emergence of the primary germ tube. The surface of the leaf remained unchanged (no epidermal collapse, discoloration, necrosis, or differences in surface wax).

A differential response was observed among the native hop botanical varieties (Table 4.2). Some genotypes of *H. l.* var. *lupuloides* genotypes showed no response to infection, while others demonstrated blistering at 72 h. Epidermal cells began to collapse after 144 h in the genotypes that showed blistering. A translucent brown

discoloration was apparent in these cells. The *H. l.* var. *pubescens* genotype exhibited no response until 120 h, when the cuticular folds (surface wax) beneath the sporeling took on a shiny, watery appearance, as if dissolved. No discoloration or apparent epidermal collapse was observed.

The three genotypes of *H. l.* var. *lupulus* behaved identically in their response (Table 4.2). After 96 h, the leaf surface under the sporeling began to blister and a golden-yellow discoloration was observed in these cells. By 120 h the epidermal cells began to collapse, after their surfaces had taken on a crystallized appearance (Fig. 4.2). The discoloration then changed to a golden-beige. Hyphae and conidiophores began to lyse, from the center of the colony outward (Figs. 4.3 (a) and (b)), in response to epidermal collapse. At this time, tiny, necrotic spots, typical of a hypersensitive response, could be seen on the leaf surface with the unaided eye. Epidermal collapse continued to radiate outwards from the center, and by 192 h, the golden-beige had turned to golden-brown. Dark brown spotting, in random epidermal cells beneath the colony, was also observed at this time (Fig. 4.4).

At 24 h after inoculation, the size of the sporelings on 'Symphony' and those on native genotypes did not differ. But by 48 h, the progression of disease on the native genotypes was approximately 24 h behind that on 'Symphony' (Table 4.3). By 48 h, the average area of colonies on 'Symphony' (0.350 mm^2), was significantly ($p < 0.01$) different than the average area (0.088 mm^2) on native genotypes ($\chi^2 = 83.96$, 1 d.f.). By 72 h, the average area of 'Symphony' had grown to 0.785 mm^2, while the area of colonies on native genotypes averaged 0.350 mm^2. After 72 hours, colonies began to overlap, which made area measurements difficult to obtain.

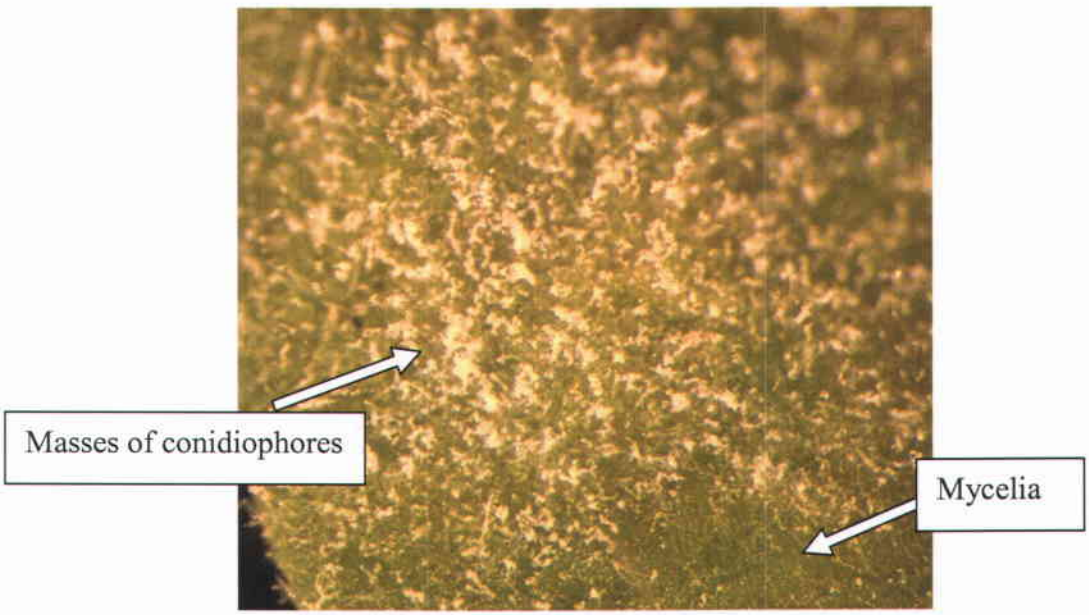

Fig. 4.1. Sporulating colony of *P. macularis* on *H. l.* 'Symphony' 10 days after inoculation.

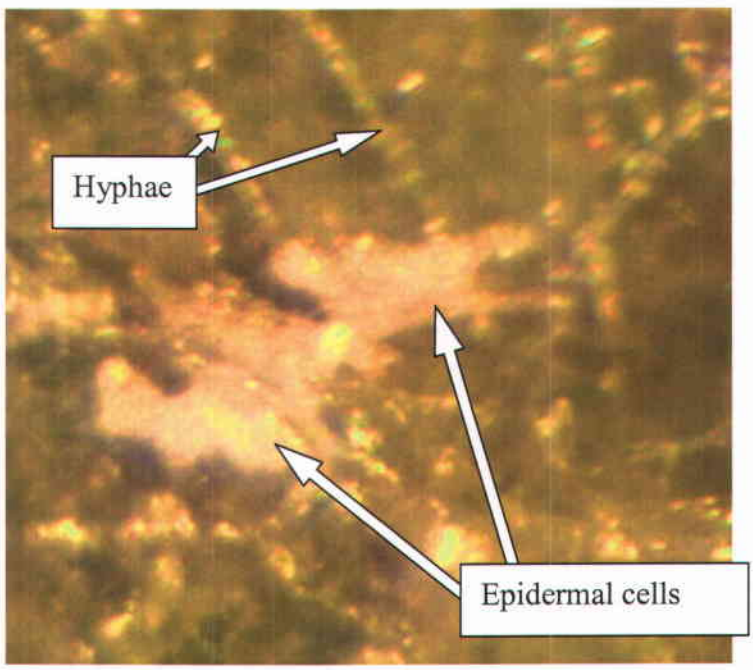

Fig. 4.2. Epidermal cell surfaces of *H. l.* var. *lupulus* demonstrating crystallized appearance after inoculation with *P. macularis*.

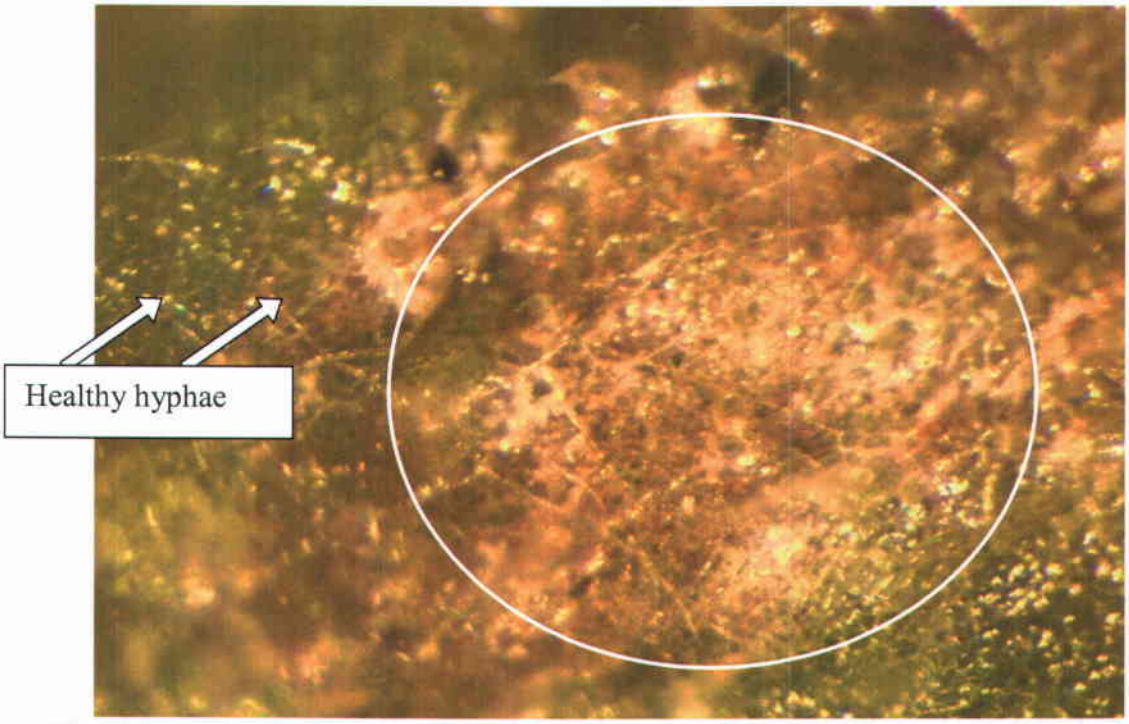

Fig. 4.3 (a). *P. macularis* hyphae and epidermal cells of *H. l.* var. *lupulus* genotypes (circled) collapse from center of colony outward.

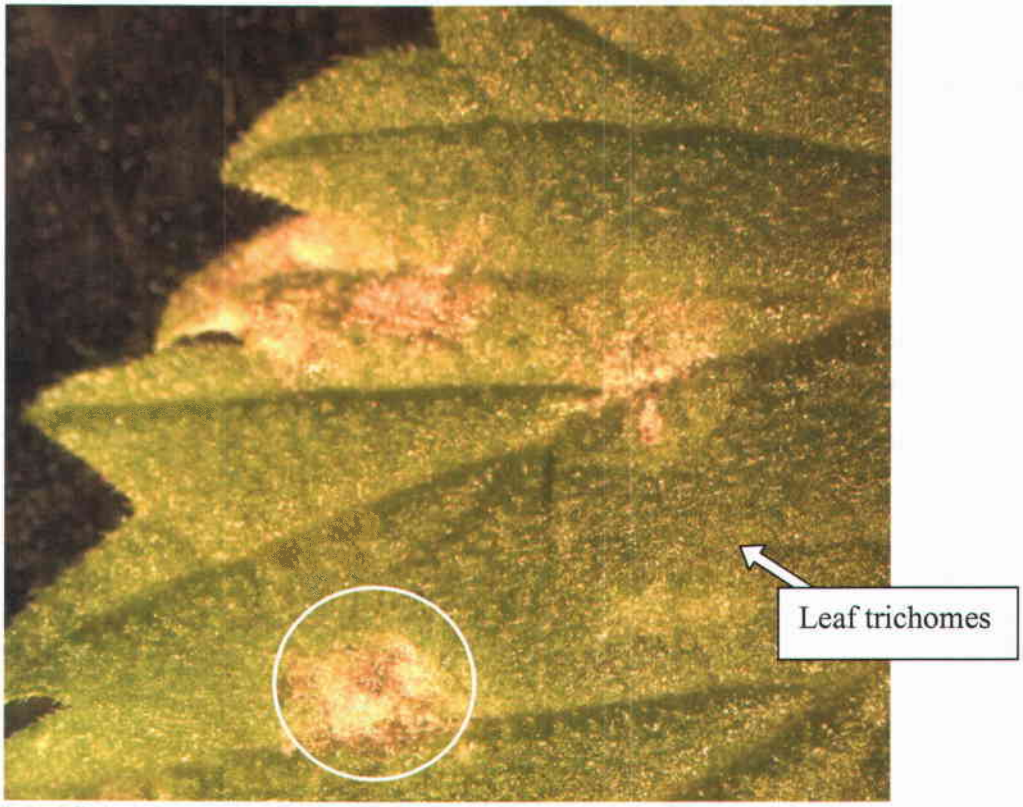

Fig. 4.3 (b). Necrotic lesions on *H. l.* var. *lupulus* genotype 10 days after inoculation with *P. macularis*. Circled region is enlarged in Fig. 4.3 (a).

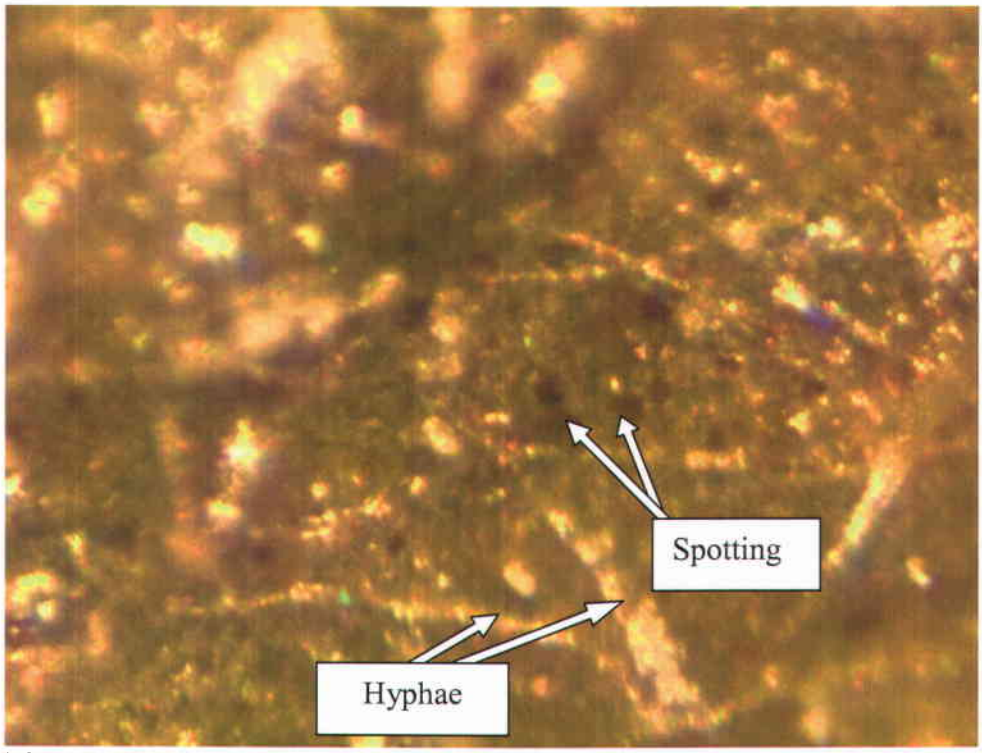

Fig. 4.4. Dark brown spotting in epidermal cells of *H. l.* var. *lupulus* genotype after inoculation with *P. macularis*.

Table 4.1. Hours after inoculation that each fungal growth stage was first observed on different hop genotypes.

Growth Stage	*H. l.* 'Symphony'	*H. l.* var. *lupuloides*	*H. l.* var. *pubescens*	*H. l.* var. *lupulus*	*H. l.* 'Nugget', 'Newport'
1 germ tube	7	15	15	15	15
2 germ tubes	24	24	24	24	-
3 germ tubes	24	48	48	72	-
4 germ tubes	48	72	72	96	-
5 germ tubes	48	96	120	96	-
1° branching	48	48	72	72	-
2° branching	96	96	120	96	-
Conidiophore Initials	96	96	144	120	-
Sporulation	120	120	168	120	-
Number of leaves observed[z]	4	24	4	12	4

[z] More than 70 conidia were observed on each leaf.

Table 4.2. Host defense response observed on hop leaves after inoculation with *P. macularis*.

Hours after Inoculation	'Symphony'[z]	*H. l.* var. *lupuloides*[y]	*H. l.* var. *pubescens*[x]	*H. l.* var. *lupulus*[w]	'Nugget' 'Newport'[v]
15	No defense response, colonies grew normally.	No Response	No Response	No Response	No obvious response – no necrosis, epidermal collapse, discoloration or difference in morphology of cuticular wax.
24					
48					
72		No Response (2 genotypes) Blistering (4 genotypes)		Blistering begins; Yellow coloration under germinated spores	
96					
120		No response or epidermal collapse with translucent-brown discoloration toward center of colony	Surface wax/cuticular folds under sporeling are dissolved (shiny, watery appearance)	Collapsing of epidermal cells; golden-beige discoloration; lysing of hyphae, tiny necrotic spots seen with unaided eye	
144					
168					
192				Epidermal collapse, golden brown discolorations; Dark brown spotting	

[z] Response from 2 plants
[y] Response from 2 plants of 6 genotypes
[x] Response from 2 plants of 1 genotype
[w] Response from 2 plants of 3 genotypes
[v] Response from 2 plants of each genotype

Table 4.3. Average area (mm^2) of *P. macularis* colonies growing on hop leaves at 18°C.

Hours after Inoculation	'Symphony'	Wild hops	'Nugget' 'Newport'
48	0.350	0.088	No Growth
72	0.785	0.350	
96	>0.785	0.785	
120		>0.785	
144			

Discussion

The infection progress of an Oregon population of *P. macularis* on 'Symphony' was similar to that of the European pathotypes on susceptible genotypes observed by Liyanage (1973, as cited by Royle, 1978), Godwin (1985), and Godwin *et al.* (1987). Germination of conidia on 'Symphony' began 8 hours earlier than on the other genotypes observed. Likewise, sporelings reached the 3rd, 4th, and 5th germ tube stage about 24 h earlier on 'Symphony', with colonies having significantly larger area than on the other genotypes by 48 h (Table 4.3).

The upper and lower epidermal cells of mature hop leaves show numerous cuticular folds covered with a continuous layer of wax devoid of any sculpturing or crystalloids. Gülz *et al.* (1993) found that removal of this layer with chloroform, ($CHCl_3$), had no effect on the cuticular foldings, demonstrating that these lamellate or undulate folds consist not of wax but of cutin. The epicuticular wax layer may provide a degree of physical defense against the ingress of fungal spores. Gülz *et al.* (1993)

suggest that the complex chemical composition of the wax layer in hops may have a specific role in protection and that a fungistatic function for leaf-wax components could be considered.

The resistant cultivar 'Nugget' possesses the R_6 gene. This resistance gene has been overcome by corresponding pathogenic strains in Germany, Belgium, France, and the United Kingdom. Although the mechanism of resistance in 'Nugget' and 'Newport' has not been characterized, observations in this study suggest that penetration by the fungus is blocked by the thickness or chemical properties of the plant cuticle, or that fungistatic conditions within host epidermal cells arrest the development of the initial haustoria. Additional studies, using electron microscopy, would further identify the mechanism.

The resistance observed in the native genotypes may be due to fungistatic chemical properties either inherent or produced in response to infection. In these genotypes, the pathogen was able to penetrate the host, but growth and subsequent sporulation was hindered. The significantly reduced area of colonies on the wild genotypes after 48 h (Table 4.3), and the collapse of epidermal cells in advance of hyphal collapse (Fig. 4.3 (a)), implies that fungistatic conditions developed in response to infection.

Haustoria in the partially resistant native genotypes may be malformed or lacking the characteristic lobes formed in the cells of susceptible genotypes. Limited fungal growth may be due to the reduced efficiency of haustoria to obtain nutrients from the

host. Additional studies using electron microscopy could evaluate the number and developmental status of haustoria in these genotypes exhibiting partial resistance.

The golden to dark brown discolorations observed in the partially resistant wild genotypes may be directly involved with resistance or may be a sign of a general stress in response to infection by the pathogen. The discolorations might indicate the presence of lignin and/or callose in the reacting epidermal cells. Godwin *et al.* (1987) identified lignin and callose in the resistant cultivar *H. l.* Wye Target, after infection with *P. macularis*.

This study has observed the differences in the growth of *P. macularis* on susceptible, resistant, and partially resistant hop genotypes, and serves as a basis for further work on control of the disease. The genes involved in the partial resistance of wild genotypes should prove useful to breeders since the selection pressure for new pathotypes of *P. macularis* to develop is reduced. The seven known major resistance genes in cultivated hops have each been overcome by more virulent strains of mildew. Partial resistance to powdery mildews, however, is general (race-nonspecific) and controlled polygenically (Bushnell, 2002). On its own, partial resistance would be useful to growers, in that the number of fungicide applications necessary for control would be considerably reduced. Since the disease was observed at the optimum temperature for fungal growth (18°C), it is expected that the partial resistance will be markedly increased under field conditions. The best strategy, however, may be to combine partial resistance with resistance controlled by major genes in a single variety (Darby *et al.*, 1989). This would reduce the selection potential for a virulent pathotype that could overcome the

major gene resistance, and should this happen, the polygenic resistance would still be operative (Neve and Darby, 1982).

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Chapter 5

Conclusion

The research presented in this thesis has identified novel powdery mildew resistant hop germplasm. The approach was to: 1) explore the Southwestern United States and collect native North American hop germplasm, 2) screen wild hop germplasm from North America and Europe for resistance to powdery mildew, 3) determine whether temperature dependent resistance occured in several hop genotypes, and 4) microscopically examine the progression of powdery mildew on various hop genotypes.

Plant Exploration

Plant collecting explorations, taken in September 2002 and 2003, obtained 60 seed and 28 plant accessions of the previously unrepresented *H. l.* var. *neomexicanus* from Colorado, New Mexico, and Arizona. In addition, herbarium specimens were collected from 9 significant new localities to be distributed to major regional herbaria. The seed and plant accessions were deposited at the USDA, ARS, National Clonal Germplasm Repository located in Corvallis, Oregon. This material should harbor significant genetic diversity and has the potential for disease and pest resistance.

Historic populations along the southern distributional range of *H. lupulus* may become extinct in the future. Loss of habitat and introgression from the naturalized *H. l.* var. *lupulus* appear to be the most significant threats to both *H. l.* var. *neomexicanus* and *H. l.* var. *pubescens*. The genetic diversity within these two botanical varieties is poorly represented in *ex situ* collections, so further exploration is recommended.

Screening of Wild Hop Germplasm

More than 2,100 wild (1563 North American and 107 Kazakhstani) hop seedlings, from 54 seedlots, were evaluated for powdery mildew resistance under greenhouse and field conditions, after inoculation with an Oregon population of *P. macularis*. Although most hop seedlings were highly susceptible to the disease, a continuum of resistance levels was also observed. A total of 32 resistant genotypes were selected for additional foliar and cone evaluation under field conditions. Foliar resistance was observed in 31 of these genotypes. Cones from 13 female and monoecious genotypes were evaluated for the presence of mildew. Those with no to low levels of cone infections include four females from Manitoba, North Dakota and Kazakhstan. These genotypes have the potential for a future germplasm release. Future studies will evaluate these genotypes for resistance to other diseases, including downy mildew (*Pseudoperonospora humuli*). Essential oil and resin analysis will evaluate their potential for use in the brewing industry.

Temperature Dependent Resistance

Studies were performed to examine the effect of temperature on host resistance. Plants were grown in growth chambers at different temperature regimes prior to inoculation with *P. macularis*. The infection frequency (lesions/cm^2) was calculated ten days after inoculation. The results demonstrate that a significant increase in resistance, or alternatively, a significant decrease in the infection frequency, is observed on susceptible to tolerant genotypes when exposed to high temperatures (>30°C) prior to inoculation.

Microscopic Examination of Disease Progression

In the final portion of this thesis, the development of *P. macularis* on several hop genotypes was observed using light microscopy. The progression of disease was followed on the commercial hop cultivars 'Symphony' (susceptible), 'Nugget' (resistant), and 'Newport' (resistant), as well as wild North American and Kazakhstani hop genotypes exhibiting polygenic resistance (reduced number of lesions, lesion size, and sporulation). Development of the disease occurred most rapidly on the highly susceptible cultivar 'Symphony'. Conidia did not progress further than the primary germ tube on 'Nugget' and 'Newport' before shriveling. The resistance observed in the native genotypes may be due to fungistatic chemical properties either inherent or produced in response to infection. In these genotypes, the pathogen was able to penetrate the host, but growth and subsequent sporulation was hindered. The significantly reduced area of colonies on the wild genotypes after 48 h, and the collapse of epidermal cells in advance of hyphal collapse, implies that fungistatic conditions developed in response to infection. The results from the temperature and disease progression studies may enhance disease forecasting models.

Based on this research, four female, two monoecious and 16 male wild hop genotypes have been identified that demonstrate high levels of resistance to powdery mildew. In my opinion, genotypes 1000.015, 1001.002, and 1001.003, from North Dakota and Manitoba, exhibit the most potential for inclusion into breeding programs. This germplasm will be a valuable genetic resource for the development of disease resistant cultivars.

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Appendix 2.1 2002 Travel Log

I= Interstate Highway US= United States Highway S= State Highway C= County Road OCJ= Collector number

Participants:
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Monday 9 September 2002
Traveled from Corvallis, OR to Aurora (Denver), CO.

Tuesday 10 September 2002
From Aurora we went west on I-70 to S-58 and west to Golden, north on S-93 to Coal Creek (OCJ-1). North on S-93 to El Dorado Springs Drive, southwest on drive to Mesa Trialhead parking lot. We found some Humulus amongst willows on the south side of Boulder creek. There were a number of male plants to the south of parking lot (OCJ-2). Continued up South Boulder Creek to Rattlesnake Gulch Trailhead (OCJ-3) and further up to El Dorado Canyon State Park (OCJ-4). Going back downstream to the rock climbing area just upstream from El Dorado Springs (OCJ-5), we made our last collection. We returned northeast back to S-93 and north to Boulder. We took US-36 north to Lyons. West of town some young plants were found with no cones. From Lyons we went on S-7 up the South St. Vrain Creek, turned onto C-103 to the small community of Raymond, to a site collected 80 years ago. We found only one plant growing on a mailbox and declined to collect it. Returned to S-7 via S-72 and went north to Estes Park. Followed US-34 down the Big Thompson River and north on US-287. Spent the night in Fort Collins.

Wednesday 11 September 2002
Stopped at Scott Dorsch's office (Busch Agricultural Resources, Inc.) to pickup shipping boxes and cone collecting buckets. From Fort Collins we went south on US-287, west on West Harmony to South Taft Hill Road, north on S. Taft Hill Rd. to West County Road 38E. Followed C-38E past Horsetooth Reservoir, up and down hill toward Redstone Creek. Halfway down the hill, we collected plants creek (OCJ-6). Followed Redstone Creek up C-N25E for about 4.5 miles to Happy Hollow Gulch (OCJ-7). Returned to C-38E and headed south to Masonville. Turned south onto C-N27 to C-W32C. Traveled up C-W32C for about a mile (no plants) and returned to Masonville. Went northwest up

Buckhorn Creek on C-N27 to Stove Prairie Road and on to S-14. Followed S-14 down the Cache La Poudre River to US-287. Went south on US-287, right on West County Road 54E, left on North County Road 25E, right on West County Road 52E, right on North County Road 27E and back to US-287 on West C-54E (no plants). Retraced route back up the Cache La Poudre River and continued on S-14 to US-40. In route, at Rustic, we turned north for a side excursion, going up C-69 then C-68C for about 6 miles. At Elkhorn Creek there was a driveway going into a ranch (locked gate). Downhill from there, in a ditch, were some *Humulus* plants. The Elkhorn drainage is large enough that there may be a number of populations. While traveling through Jackson County, it appeared too cold for *Humulus* to grow. We went west on US-40 to Rabbit Ears Pass (OCJ-8) and on to Steamboat Springs for the night.

Thursday 12 September 2002

Traveled west from Steamboat Springs on US-40 to Milner (OCJ-9) and the area around Hayden (OCJ-10). Explored north and east on C-80 for 2 or 3 miles and returned back to Hayden. We went back eastward on US-40, north on C-52 going along Wolf Creek. Turned around at Meadow Gulch (OCJ-11) and returned to US-40. Going westward, we turned right onto C-70 for only 100m (OCJ-12) and backtracked to US-40. Went west on US-40 for a mile and turned south on C-27 for just a mile (no cones on plants) before taking US-40 to the west of Hayden (OCJ-13). Turned south on S-13 passing though Hamilton and going by Axial along Good Spring Creek up to about milepost 60 (OCJ-14 to OCJ-17) before returning to the westward trek on US-40. At Maybell we turned northwest onto S-318 going to its end at the Utah boarder (OCJ-18). After returning to US-40 we continued west to Vernal, Utah for the night.

Friday 13 September 2002

From Vernal we went eastward on US-40 back to State highway 64 at Dinosaur. Followed S-64 to Rangley, continued east, out of town to C-122, and followed it south to Calamity Ridge (we had a flat tire in route). No plants were found. Went downhill, eastward on C-122 then C-20 to C-5 and finally back to S-64. We traveled on S-64 and S-13 to Meeker. To the east of town we turned right onto C-8 and followed the White River. Turned south on C-57, and went up Miller Creek 4.5 miles (OCJ-19 and OCJ-20). Returned to C-8 and went east to Buford, turned south on New Castle Buford Road. Turned up C-10, following the South Fork of the White River to the South Fork Campground (OCJ-21). Returned to Buford and went just east on C-8 past the buildings (OCJ-22). Returned to S-13 and traveled south to I-70 and on to Glenwood Springs for the night.

Saturday 14 September 2002

From Glenwood Springs we traveled back up S-13 to Rio Blanco, west on C-5 for 6 miles to Deer Gulch, and went up a small overgrown dirt road (OCJ-23 to OCJ-25). Back on C-5 we went 1.5 miles toward S-13, where a plant with cones was found along Piceance Creek (OCJ-26). Followed S-13 toward I-70 and turned north on S-325 to Rifle Gap (no cones on plants). From Rifle Gap Reservoir, we explored up S-325 for seven miles (OCJ-27, OCJ-28) and C-252 for the same (no plants). From S-325, via I-70 we went north on the Colorado River Road to Sweetwater Road and followed it to Sweetwater Lake (OCJ-29

to OCJ-31). Returned to I-70 and went east to Vail Pass and up Shrine Pass Road to summit (OCJ-32). Spent the night further east, down I-70 at Frisco.

Sunday 15 September 2002
From Frisco we went east on I-70 to Silver Plume (OCJ-33). From here we went back westward on I-70 and south on S-91 and US-24, then west on S-82 to Aspen (OCJ-34). We continued westward on S-82 and south on S-133 to Paonia. From Paonia, we took back roads and tracks toward Lamborn Mountain and Landsend Peak, but daylight ran out on us (no plants found). After returning toward Paonia we followed C-39.00 to S-92 which lead us to US-50 and east to Gunnison for the night.

Monday 16 September 2002
On this day Scott Dorsch (Busch Agricultural Resources Inc.) and Nazareno Perez (Agrar del Sur) joined us to collect plants. From Gunnison, we went west on US-50, south on S-149, south on C-31, and went up Willow Creek to where Pole Creek enters (OCJ-35 to OCJ-39). Back on US-50, we went east of Gunnison, turned south on S-114 and up Cochetopa Creek for 5 miles to just before C-43A (OCJ-40). Continued on US-50 eastward to where Owens Creek enters Tomichi Creek, or roughly 6.5 miles west of Sargents (OCJ-41). Followed US-50 east, went north past Nathrop on US-285 to C-301. On C-301A between US-285 and the Arkansas River, we found *Humulus* (OCJ-42). Went south on US-285, past US-50, and went up Poncha Creek for about 5 miles (OCJ-43). Continued south on US-285, took S-17 south to Lane 6 N and went east to S-150 into the Great Sand Dunes National Monument. Explored up Mosca Creek (no plants). Scott Dorsch agreed to ship the samples we had collected so far back to NCGR-Corvallis. Spent the night in Alamosa.

Tuesday 17 September 2002
In the morning we went east on US-160 up Sangre de Cristo Creek, past Fort Garland to Cottonwood Gulch (OCJ-44) and to where Malo Vega Creek enters (OCJ-45) before turning back westward. West of Alamosa, we took US-160 to Monte Vista, south on S-15, west on C-E2S, south and west on C-28 up Rock Creek. On C-28, 1.5 miles west of the junction with C-W9S (OCJ-46) and at Burnt Gulch (OCJ-47) plants with cones were found. Returned to US-160, going west to S-149, and up the Rio Grande River to Wagon Wheel Gap (OCJ-48). Back on US-160, we continued west to Devil Creek (OCJ-49) near Chimney Rock and the Piedra River. We then went west on US-160 and north on US-550 to Ouray for the night.

Wednesday 18 September 2002
Traveled north from Ouray on US-550, westward on S-62 to Leopard Creek. At about 2.5 miles (OCJ-50) and one-forth mile (OCJ-51) from Placerville and the junction with S-145, collections were made. We continued south on S-145 to where the West Delores River joins the Delores River (OCJ-52) and onward to US-160. From here we went eastward on US-160 to Durango. At Cherry Gulch (OCJ-53), about 5 miles west of Durango cones were collected. South from Durango, on US-550, S-544 and S-44 we traveled into New Mexico crossing I-25 onto S-165 which turns into S-44. We followed Los Huertas Creek (OCJ-54) and found *Humulus*. Traveled north on I-25 and spent the night in Santa Fe.

Thursday 19 September 2002

From Santa Fe, we took I-25 eastward to S-50, and then north on S-63 along the Pecos River. Collections were made between the Windy Bridge and the Bert Clancy recreation sites (OCJ-55 to OCJ-60). Backtracked to I-25, and went north of Las Vegas on S-518 and northwest on S-94 to Manuelitas Creek (OCJ-61). We continued northwest up S-518 to S-68 which connected to US-64. Traveled eastward on US-64 past Eagle Nest to the Cimarron River (OCJ-62, OCJ-63). The upper reaches of the Cimmaron River had many *Humulus* visible from the road. We returned to Colorado, taking US-64 and I-25. Just over the boarder, at Raton Creek, a collection was made (OCJ-64). Further north on I-25 we went 19 miles west of Trinidad on S-12, to the last site of the day and collected by flashlight (OCJ-65). The last night was spent at Pueblo.

Friday 20 September 2002

Took US-50 west out of Pueblo. Before reaching Canon City, we turned north on Phantom Canyon Road (C-67), east on C-123 and north on Upper Beaver Creek Road (C-132) to its end on Beaver Creek (OCJ-66). We returned to Phantom Canyon Road and followed it up Eight Mile Creek passed the second tunnel (OCJ-67 to OCJ-74). Returned to US-50 and went east, then north on S-115 to I-25. Traveled north on I-25 to Castle Rock, turned west on US-85 to Sedalia. Turned southwest on S-67 and went less than a mile to West Plum Creek (OCJ-75). From here we drove directly to the Denver International Airport for the flight to Corvallis, Oregon.

Appendix 2.2. 2003 Travel Log

I = Interstate Highway US = United States Highway S = State Highway
FR = Forest Service Road OJ = Collector Number

Participants:
Jodi M. Smith, Biological Sciences Research Technician, USDA ARS NCGR-Corvallis and Graduate Research Assistant, Oregon State University, Department of Horticulture

James M. Oliphant, Biological Science Research Technician, USDA ARS NCGR-Corvallis, 33447 Peoria Road, Corvallis, Oregon, 97333-2521

Monday 8 September 2003

Traveled from Portland, Oregon to Phoenix, Arizona.

Tuesday 9 September 2003

From Phoenix, headed NW on US-60 to Wickenburg. Took US-93 to S-89 to Kirkland Junction. Followed Zonia Mine Road to Placerita and explored along Arrastre Creek. Although the habitat looked suitable for hops, none were found. Took S-89 to Centerville and followed an unmarked FR to Sycamore Canyon Wilderness. Hiked Pack Trail in to where Summers Spring flows into Sycamore Creek. Habitat looked suitable for hops, but none were found. Spent night in Cottonwood.

Wednesday 10 September 2003

From Cottonwood, headed NW on Alt. S-89 to Oak Creek Canyon. Hops were found growing along the highway, above a creek, north of Cave Springs. A few seeds were found to be mature, so cones were collected (OJ-01). Continued on Alt. S-89 to Flagstaff and followed Lake Mary Road towards Lower Lake Mary. Hops were found along Walnut Creek, below the road at the mouth of Lower Lake Mary. No ripe seed was found, so cuttings were collected (OJ-02). Continued on Lower Lake Mary Road. Headed NE on S-87. Took FR-137 to Mack's Crossing. Hops were found along East Clear Creek, 1 mi. S of S-87 on FR-137. Cones were collected (OJ-03). Drove S-87 to Payson for the night.

Thursday 11 September 2003

From Payson headed East on S-260 to Show Low. Explored area around Show Low Lake. Habitat was not found to be suitable. Continued on S-260 to McNary. Found hops growing with willow (*Salix sp.*) along the North Fork of the White River at junction with S-473. Cones were collected (OJ-04). Continued on S-260 to Springerville and headed S on US-180. Took FR-281 (Blue River Road) and followed the Blue River. Although the habitat looked suitable for hops (riparian habitat with willow present), no hops were found. Took S-191 N to Springerville for the night.

Friday 12 September 2003

From Springerville, headed S on S-180 towards Alpine. Hops were found growing with willow along a perennial creek, below S-180 about 7 mi. W of Alpine, along the San

Francisco River, just W of mile post 420. The large, well-seeded cones were collected (OJ-05). Continued along S-180, entering NM. Traveled E on S-12 to Reserve. Headed S on S-435 to Willow Springs Canyon. Habitat could have been suitable to hops in the past, but has since been heavily grazed and no hops were found. Backtracked to Reserve and headed NE on S-12 to Apache Creek. Took FR-94 S to FR-28. Traveled FR-28 to S-159. Headed S on S-159 to Gilita Creek Campground. Found hops growing on rocks near perennial creek. Cones were collected (OJ-06). Continued on S-159 to Glenwood. At Glenwood took US-180 S to S-78. Followed S-78 W into AZ to US-191. Traveled US-191 to I-10. Headed W on I-10 to Tucson for the night.

Saturday 13 September 2003

From Tucson, took Catalina Highway N to Mount Lemmon in the Santa Catalina Mountains. Found hops along a perennial stream in a willow thicket on Sabino Canyon Road, south of Summerhaven, 0.7 mi. from Ski Run Road. No seed was observed so cuttings from 2 females were collected (OJ-07). Backtracked to Tucson and took I-10 E to Wilcox for the night.

Sunday 14 September 2003

From Wilcox, took I-10 E to San Simon-Paradise Road. Headed S to Foothills Road and followed it to Portal. Took FR-42 from Portal SW into the Chiricahua Mountains to FR-610. Followed FR-610 to turn around area at Herb Martyr Campground. Hiked along Cima Creek to Winn Falls. Ascended gorge and followed trail back to campground. No hops were found. Backtracked to Portal and took Portal Road to S-80. Headed N on S-80 to I-10. Went W on I-10 to Wilcox for the night.

Monday 15 September 2003

From Wilcox, took S-186 to S-181. From S-181 headed SE on Pinery Canyon Road (FR-42) into the Chiricahua Mountains. Took FR 420 to Buena Vista Peak campground. Hiked trail to Winn Falls. Collected fruit from one plant of *Ribes pinetorum* (OJ-08) along Crest Trail S of Flys Peak. Collected plants from one clone of *Fragaria virginiana* (OJ-09) from shaded riparian forest area along Greenhouse Trail. Backtracked and hiked Crest Trail to Chiricahua Peak. Collected fruit from many plants of *Rubus strigosus* (OJ-10) along Crest Trail. Collected plants from one clone of *Fragaria virginiana* (OJ-11) along Crest Trail in shrub area along ridge in burned forest. Backtracked to car and followed exact route back to Wilcox for the night.

Tuesday 16 September 2003

From Wilcox, headed W, entering NM on I-10. At Lordsburg took S-90 to Silver City. Took S-152 to Hillsboro. Explored area around Hillsboro and found no suitable habitat for hops. Continued E on S-152 to I-25. Followed I-25 S to Las Cruces and took US-70 NE to Alamogordo for the night.

Wednesday 17 September 2003

From Alamogordo headed E on US-82 to Karr Canyon Road. Explored Karr Canyon to junction with FR-623. Although habitat appeared suitable, no hops were found. Backtracked to Alamogordo and headed N on US-70/US-54 to Tularosa. Followed US-70

to Hollywood. Took US-48 N to US-532 and headed W. Found one female hop clone growing on dead *Sambucus sp.* along highway, below mile post 7 in Carlton Canyon. No seeds were found so cuttings were collected (OJ-12). Another hop clone was found along the highway, above mile post 7 at Windy Point. No seeds were found, so rhizomes were collected (OJ-13). Continued W on highway to Sierra Blanca Ski area. Plants were collected from one clone of *Fragaria virginiana* (OJ-14) along the N Fork of the Ruidoso River, above ski area. Backtracked towards Ruidoso. Traveled road leading to Monjeau Peak Lookout. No hops were found. Backtracked to Ruidoso. Took S-48 N to S-37 N to US-380. Followed US-380 NW to San Antonio. Headed N on I-25 to Socorro. Took US-60 W to FR-549. Headed S into Withington Wilderness in the San Mateo Mountains. Found hops in Bear Trap Canyon, 4.8 mi. SW of Hughes Campground on FR-549. No seeds were found, so cuttings were taken from one female, one male and one of unknown sex (OJ-15). Backtracked to Socorro and headed S on I-25 to Las Cruces. Headed W on I-10 to Deming for the night.

Thursday 18 September 2003

From Deming, headed W on I-10, N on US-191 to Safford, AZ. From Safford, took Frye Mesa Road (FR-103) up into Frye Canyon in the Pinaleno Mountains. Hiked trail and found one female hop clone growing on rocks in a dry, shaded stream bed. Rhizomes were collected (OJ-16) since no seed was found. Backtracked to Safford and headed S on S-191. Headed SW on S-366 (Swift Trail Parkway). Hops were found growing along the road below Show Flat Road in the Pitchfork Canyon drainage. Cones, rhizomes and cuttings were collected from one female and rhizomes and cuttings were collected from one male (OJ-17). Hiked to the lookout tower of Heliograph Peak in search of *Vaccinium myrtillus*. Backtracked to Safford and headed NW on US-70 to Globe for the night.

Friday 19 September 2003

From Globe, headed W on US-60 to Phoenix. Flew to Portland, Oregon.

Sunday 12 October 2003

From North Rim headed north on S-67 (North Rim Parkway), then headed NE on FR 422 (West Side Road), turned east on FR 429. After 0.4 miles turned south and followed road along creek, just before Riggs Spring found one female hop clone growing on east-facing rocks (OJ-19). Returned to FR 422 and at Ryan turned east onto FR 462. At Mud Lake, turned east onto FR 212 and followed road until junction with S-67. Collected *Fragaria virginiana* (OJ-20) at junction.

Appendix 2.3. 2002 Collections of *Humulus lupulus var. neomexicanus* grouped by watershed.

Watershed	Location Name	Latitude	Longitude	Elevation (m)	Form	Amount	Herbarium	Date	Collection #	Local #
Arkansas R.	Nathrop	38.414	-106.097	2348	SD	22,380		9/16/02	OCJ-42	CHUM 1370
Arkansas R.	Poncha Creek	38.455	-106.101	2532	SD	21,500	1 sheet	9/16/02	OCJ-43	CHUM 1371
Arkansas R.	Phantom Canyon 1	38.569	-105.015	1872	SD	3235		9/20/02	OCJ-66	CHUM 1393
Arkansas R.	Phantom Canyon 2	38.550	-105.100	2088	PL	1	2 sheets	9/20/02	OCJ-68	CHUM 1395
Arkansas R.	Phantom Canyon 2	38.550	-105.100	2088	PL	1		9/20/02	OCJ-69	CHUM 1396
Arkansas R.	Phantom Canyon 2	38.550	-105.100	2088	PL	1		9/20/02	OCJ-70	CHUM 1397
Arkansas R.	Phantom Canyon 2	38.550	-105.100	2088	PL	1		9/20/02	OCJ-71	CHUM 1398
Arkansas R.	Phantom Canyon 2	38.550	-105.100	2088	PL	1		9/20/02	OCJ-72	CHUM 1399
Canadian R.	Manuelitas	35.809	-105.288	2176	SD	21,650		9/19/02	OCJ-61	CHUM 1388
Canadian R.	Cimarron R. 1	36.538	-105.227	2533	SD	100		9/19/02	OCJ-62	CHUM 1389
Canadian R.	Cimarron R. 2	36.537	-105.206	2455	SD	13,565		9/19/02	OCJ-63	CHUM 1390
Colorado R.	East Rifle Creek 1	39.648	-107.708	1887	SD	19,000		9/14/02	OCJ-27	CHUM 1357
Colorado R.	East Rifle Creek 2	39.690	-107.703	2009	SD	20,965		9/14/02	OCJ-28	CHUM 1358
Colorado R.	Sweetwater Lake 1	39.810	-107.182	2366	SD	9640		9/14/02	OCJ-29	CHUM 1359
Colorado R.	Sweetwater Lake 2	39.810	-107.171	2363	SD	28,180		9/14/02	OCJ-30	CHUM 1360
Colorado R.	Sweetwater Lake 3	39.798	-107.161	2363	SD	4900		9/14/02	OCJ-31	CHUM 1361
Colorado R.	Aspen	39.185	-106.808	2437	SD	5760		9/15/02	OCJ-34	CHUM 1363
Dolores/San Miguel R.	Leopard Creek 1	38.049	-108.035	2300	SD	6700	2 sheets	9/18/02	OCJ-50	CHUM 1378
Dolores/San Miguel R.	Leopard Creek 2	38.023	-108.055	2228	SD	7900		9/18/02	OCJ-51	CHUM 1379
Dolores/San Miguel R.	Dolores River	37.590	-108.499	2265	SD	80		9/18/02	OCJ-52	CHUM 1380
Gunnison R.	Willow Creek 1	38.453	-107.058	2357	SD	890		9/16/02	OCJ-35	CHUM 1364
Gunnison R.	Willow Creek 2	38.453	-107.058	2342	PL	3	1 sheet	9/16/02	OCJ-36	CHUM 1365
Gunnison R.	Willow Creek 2	38.453	-107.059	2342	PL	1		9/16/02	OCJ-37	CHUM 1366
Gunnison R.	Willow Creek 2	38.453	-107.059	2342	PL	1		9/16/02	OCJ-38	CHUM 1367
Gunnison R.	Cochetopa Canyon	38.458	-106.758	2439	SD	46,000	3 sheets	9/16/02	OCJ-40	CHUM 1368
Gunnison R.	Tomichi Creek	38.414	-106.512	2518	SD	2000		9/16/02	OCJ-41	CHUM 1369
Pecos R.	Pecos 1	35.717	-105.680	2328	SD	16		9/19/02	OCJ-56	CHUM 1383
Pecos R.	Pecos 1	35.717	-105.680	2328	PL	6		9/19/02	OCJ-57	CHUM 1384
Pecos R.	Pecos 1	35.717	-105.680	2328	PL	1		9/19/02	OCJ-58	CHUM 1385
Pecos R.	Pecos 2	35.737	-105.678	2354	SD	365		9/19/02	OCJ-59	CHUM 1386
Pecos R.	Pecos 2	35.737	-105.678	2354	PL	5		9/19/02	OCJ-60	CHUM 1387

Appendix 2.3. 2002 Collections of *Humulus lupulus var. neomexicanus* grouped by watershed (Continued).

Watershed	Location Name	Latitude	Longitude	Elevation (m)	Form	Amount	Herbarium	Date	Collection #	Local #
Purgatoire R.	Wooton	37.019	-104.491	2201	SD	7000		9/19/02	OCJ-64	CHUM 1391
Purgatoire R.	Cordova Plaza	37.134	-104.815	2070	SD	17,590		9/19/02	OCJ-65	CHUM 1392
Rio Grande R.	Sangre de Cristo 1	37.495	-105.334	2409	SD	2565		9/17/02	OCJ-44	CHUM 1372
Rio Grande R.	Sangre de Cristo 2	37.531	-105.295	2541	SD	6940		9/17/02	OCJ-45	CHUM 1373
Rio Grande R.	Rock Creek 1	37.495	-106.236	2484	SD	3765		9/17/02	OCJ-46	CHUM 1374
Rio Grande R.	Rock Creek 2	37.490	-106.260	2530	SD	3100		9/17/02	OCJ-47	CHUM 1375
Rio Grande R.	Wagon Wheel Gap	37.769	-106.800	2537	SD	16,075		9/17/02	OCJ-48	CHUM 1376
Rio Grande R.	Las Huertas Creek	35.250	-106.411	2149	SD	7880		9/18/02	OCJ-54	CHUM 1382
San Juan/Animas R.	Chimney Rock	37.213	-107.298	2025	SD	6895		9/17/02	OCJ-49	CHUM 1377
San Juan/Animas R.	Cherry Gulch	37.274	-107.960	2164	SD	9080	2 sheets	9/18/02	OCJ-53	CHUM 1381
South Platte R.	Coal Creek	39.916	-105.237	1829	SD	1830		9/10/02	OCJ-1	CHUM 1333
South Platte R.	El Dorado Springs 1	39.939	-105.258	1723	SD	3500		9/10/02	OCJ-2	CHUM 1334
South Platte R.	El Dorado Springs 2	39.931	-105.291	1890	SD	4700		9/10/02	OCJ-3	CHUM 1335
South Platte R.	El Dorado Springs 3	39.930	-105.297	1890	SD	1600		9/10/02	OCJ-4	CHUM 1336
South Platte R.	El Dorado Springs 4	39.931	-105.282	1832	SD	10,360		9/10/02	OCJ-5	CHUM 1337
South Platte R.	Redstone Creek 1	40.515	-105.188	1722	SD	7785		9/11/02	OCJ-6	CHUM 1338
South Platte R.	Redstone Creek 2	40.567	-105.230	1813	SD	5320		9/11/02	OCJ-7	CHUM 1339
South Platte R.	Silver Plume	39.697	-105.724	2791	SD	17,940		9/15/02	OCJ-33	CHUM 1362
South Platte R.	Sedalia/W. Plum Cr.	39.429	-104.969	1779	SD	12,910		9/20/02	OCJ-75	CHUM 1402
White R.	Miller Creek	39.882	-107.768	2192	SD	35,090		9/13/02	OCJ-19	CHUM 1350
White R.	South Fork Canyon	39.868	-107.535	2338	SD	35,000		9/13/02	OCJ-21	CHUM 1351
White R.	Buford	39.989	-107.615	2157	SD	56,145		9/13/02	OCJ-22	CHUM 1352
White R.	Deer Gulch 1	39.770	-108.003	2179	PL	2		9/14/02	OCJ-23	CHUM 1353
White R.	Deer Gulch 1	39.770	-108.003	2179	PL	2		9/14/02	OCJ-24	CHUM 1354
White R.	Deer Gulch 1	39.770	-108.003	2179	PL	2		9/14/02	OCJ-25	CHUM 1355
White R.	Deer Gulch 2	39.765	-108.014	2079	SD	51	2 sheets	9/14/02	OCJ-26	CHUM 1356
Yampa R.	Milner	40.487	-107.089	2063	SD	25,980	3 sheets	9/12/02	OCJ-9	CHUM 1340
Yampa R.	Hayden E	40.488	-107.159	1975	SD	7740		9/12/02	OCJ-10	CHUM 1341
Yampa R.	Wolf Creek	40.509	-107.131	2032	SD	715		9/12/02	OCJ-11	CHUM 1342
Yampa R.	Hayden E2	40.489	-107.156	1964	SD	5850		9/12/02	OCJ-12	CHUM 1343

Appendix 2.3. 2002 Collections of *Humulus lupulus var. neomexicanus* grouped by watershed (Continued).

Watershed	Location Name	Latitude	Longitude	Elevation (m)	Form	Amount	Herbarium	Date	Collection #	Local #
Yampa R.	Hayden W	40.492	-107.299	1939	SD	22,640		9/12/02	OCJ-13	CHUM 1344
Yampa R.	Axial 1	40.263	-107.789	1994	SD	14,800		9/12/02	OCJ-14	CHUM 1345
Yampa R.	Axial 2	40.254	-107.788	2014	SD	21,670		9/12/02	OCJ-15	CHUM 1346
Yampa R.	Axial 3	40.247	-107.785	2025	SD	3900		9/12/02	OCJ-16	CHUM 1347
Yampa R.	Axial 4	40.270	-107.791	1987	SD	7200		9/12/02	OCJ-17	CHUM 1348
Yampa/Green R.	Beaver Creek	40.866	-109.024	1733	SD	5220	2 sheets	9/13/02	OCJ-18	CHUM 1349

Appendix 2.4. 2003 Collection of *Humulus lupulus* var. *neomexicanus*

Location	County/State	Form	Sex	Amount	Latitude	Longitude	Elevation (m)	Date	Collection #	Local #
Oak Creek Canyon	Coconino, AZ	SD	n/a	1000	35.023	-111.736	1713	9/10/2003	OJ-01	1424
Walnut Creek	Coconino, AZ	PL	F	1	35.115	-111.588	2080	9/10/2003	OJ-02	1425
Mack's Crossing	Coconino, AZ	SD	n/a	1000	34.619	-111.093	1916	9/10/2003	OJ-03	1426
McNary	Apache, AZ	SD	n/a	1000	34.046	-109.728	2370	9/11/2003	OJ-04	1427
Alpine	Apache, AZ	SD	n/a	1000	33.922	-109.183	2441	9/12/2003	OJ-05	1428
Gilita Creek	Catron, NM	SD	n/a	1000	33.410	-108.574	2404	9/12/2003	OJ-06	1429
Mount Lemmon	Pima, AZ	PL	F	1	32.436	-110.759	2306	9/13/2003	OJ-07	1430
Mount Lemmon	Pima, AZ	PL	F	1	32.436	-110.759	2306	9/13/2003	OJ-07	1431
Carlton Canyon	Lincoln, NM	PL	F	1	33.396	-105.757	2646	9/17/2003	OJ-12	1432
Windy Point	Lincoln, NM	PL	F	1	33.406	-105.757	2798	9/17/2003	OJ-13	1433
Bear Trap Canyon	Socorro, NM	PL	F	1	33.810	-107.587	2303	9/17/2003	OJ-15	1434
Bear Trap Canyon	Socorro, NM	PL	M	1	33.810	-107.587	2303	9/17/2003	OJ-15	1435
Bear Trap Canyon	Socorro, NM	PL	n/a	1	33.810	-107.587	2303	9/17/2003	OJ-15	1436
Frye Canyon	Graham, AZ	PL	F	1	32.730	-109.855	1965	9/18/2003	OJ-16	1437
Pitchfork Canyon	Graham, AZ	PL	F	1	32.644	-109.851	2697	9/18/2003	OJ-17	1438
Pitchfork Canyon	Graham, AZ	PL	M	1	32.644	-109.851	2697	9/18/2003	OJ-17	1439
Pitchfork Canyon	Graham, AZ	SD	n/a	1000	32.644	-109.851	2697	9/18/2003	OJ-17	1440
Lookout Canyon	Coconino, AZ	PL	F	1	36.582	-112.344	2174	10/12/2003	OJ-19	1441

Appendix 3.1. Greenhouse and field assessment of powdery mildew on 29 native North American and 3 native Kazakhstani hop genotypes. Field planted June 2003 and field inoculated August 2004 (15,250 spores/ml).

	Genotype	Sex	2001 GH Assessment	2004 Field Leaf Assessment	2004 Field Cone Assessment[1]
1	1000-01	M	Tolerant - Low sporulation	No mildew	n/a
2	1000-07	M	Tolerant - Low sporulation	No mildew	n/a
3	1000-13	M	No mildew - repotted	No mildew	n/a
4	1000-17	F	Tolerant - Low sporulation	No mildew	81/100 (81%); 49/50 (98%); most hyphae & conidiophores misshapen/lysed
5	1000-24	F	A few colonies, on side arms with low sporulation	No mildew	0/100 (0%)
6	1000-32	M	A few colonies, rapid death of colonies	No mildew	n/a
7	1000-36	F	A few colonies - low sporulation	No mildew	0/2 (0%) only 2 cones, small plant
8	1000-37	F	A few colonies - no active sporulation, colonies appear dead	No mildew	26/50 (52%); 62/100 (62%)
9	1000-39	M	No mildew	2 leaves w/ HR[2] after inoculation	n/a
10	1000-42	M	Several areas of off color - microscop. Aborted infections	A few leaves w/ HR	n/a
11	1000-45	M	Several areas of off color - microscop. Aborted infections	No mildew	n/a
12	1000-47	F	No mildew	No mildew	no cones '04
13	1001-02	F	Tolerant Chlorotic colonies w/ low sporulation	No mildew	0/100 (0%); 7/100 (7%)
14	1001-03	F	Tolerant - no new infections, old ones w/ low sporulation	No mildew	4/26 (15%) only seen w/scope; 3/100 (3%) no visible signs minor discoloration on a few bracts
15	1001-10	M	Tolerant-Chlorotic small colonies - low sporulation	No mildew	n/a
16	1001-16	M	Tolerant-Chlorotic small colonies - low sporulation	No mildew	n/a
17	1001-19	M, F'04	Tolerant-Chlorotic small colonies - low sporulation	No mildew	100/100 (100%) no visible signs except light brown spotting, conidiophores seen w/scope
18	1001-20	M	Tolerant-Chlorotic small colonies - low sporulation	No mildew	n/a
19	1001-21	M	Tolerant-Chlorotic small colonies - low sporulation	No mildew	n/a
20	1001-24	F, M'04	Tolerant Chlorotic small colonies - low sporulation	No mildew	0/62 (0%)

21	1001-34	M	Tolerant - Low sporulation	No mildew	n/a
22	1001-36	M+F	Tolerant - Low sporulation	No mildew	12/50 (24%) only seen w/scope 2 other fungi present - black sporulating & thin white mycelia no spores (not powdery mildew)
23	1002-26	F, M'04	Tolerant - Low sporulation	No mildew	0/100 (0%); 4/100 (4%) more F than M flowers
24	1005-10	M	Moderate -chlorotic colonies w/ low sporulation	No mildew	n/a
25	1008-04	M	Tolerant -small colonies w/ med. sporulation	No mildew	n/a
26	1008-05	M	Tolerant-small colonies w/ med. Sporulation	No mildew	n/a
27	1008-19	M	No mildew	No mildew	n/a
28	1019#1-45	F	Tolerant-a few chlorotic colonies w/low spor. Most appear dead	Mildew 9/3/04 Sm. sporulating colonies	48/50 (96%); 13/18 (72%)
29	1019#5,6-05	F	No mildew - repotted	No mildew	92/100 (92%);12/20 (60%) tiny brown flecks on cones
30	1025-01		9/25/02 no mildew	No mildew, HR seen after inoculation	No flowers
31	1025-10		9/25/02 no mildew	No mildew, HR seen after inoculation	No flowers
32	1025-18	F	9/25/02 no mildew	No mildew, HR seen after inoculation	0/38 (0%) very small cones

[1] Clonal replicate data included
[2] HR = Hypersensitive Response

Appendix 3.2 Locality information for each seedlot screened for powdery mildew resistance.

Local #	Seedlot	Botanical Variety	Origin	Latitude	Longitude	Elevation (m)
804	KAZ 93-05-02	*lupulus*	Taldygorghan, Kazakhstan	45.407	80.408	1260
1000	Souris-E2	*lupuloides*	Manitoba, Canada	49.955	-100.240	420
1001	Logan-N	*lupuloides*	North Dakota, U.S.	48.160	-101.161	490
1002	Burlington-N	*lupuloides*	North Dakota, U.S.	48.282	-101.433	487
1003	Burlington-N#2	*lupuloides*	North Dakota, U.S.	48.296	-101.458	488
1004	Minot-E	*lupuloides*	North Dakota, U.S.	48.224	-100.263	487
1005	White Earth-S	*lupuloides*	North Dakota, U.S.	48.330	-102.760	639
1006	White Earth-S2	*lupuloides*	North Dakota, U.S.	48.313	-102.765	635
1007	Little Knife-E	*lupuloides*	North Dakota, U.S.	48.135	-102.440	649
1008	Oxbow-S	*lupuloides*	Saskatchewan, Canada	49.219	-102.183	500
1009	Indian Head-N	*lupuloides*	Saskatchewan, Canada	50.634	-103.562	525
1010	Bridge 2S	*lupuloides*	Saskatchewan, Canada	50.610	-103.560	520
1011	2 Qu'Appelle	*lupuloides*	Saskatchewan, Canada	50.561	-103.330	520
1012	3 Qu'Appelle	*lupuloides*	Saskatchewan, Canada	50.531	-103.258	520
1013	Grenfell-N	*lupuloides*	Saskatchewan, Canada	50.636	-102.880	480
1014	Melville-S	*lupuloides*	Saskatchewan, Canada	50.644	-102.823	480
1015	4 Qu'Appelle	*lupuloides*	Saskatchewan, Canada	50.539	-102.478	470
1017	Lisbon-NW	*lupuloides*	North Dakota, U.S.	46.461	-97.723	341
1018	Fort Ransom	*lupuloides*	North Dakota, U.S.	46.546	-97.930	354
1019	Rulo-E	*pubescens*	Missouri, U.S.	40.061	-95.393	260
1020	Rulo-E2	*pubescens*	Missouri, U.S.	40.069	-95.366	260
1024	KAZ-067	*lupulus*	Kazakhstan	48.889	58.572	394
1025	KAZ-098	*lupulus*	Kazakhstan	49.165	58.686	403
1042	Foxholm-N #1	*lupuloides*	North Dakota, U.S.	48.377	-101.582	519
1043	MP73-W #1	*lupuloides*	North Dakota, U.S.	48.403	-101.634	518
1044	MP73-W #2	*lupuloides*	North Dakota, U.S.	48.403	-101.634	518
1045	Carpio-S #1	*lupuloides*	North Dakota, U.S.	48.439	-101.724	541
1333	Coal Creek	*neomexicanus*	Colorado, U.S.	39.916	-105.239	1829
1334	Mesa Trailhead	*neomexicanus*	Colorado, U.S.	39.939	-105.258	1723

Appendix 3.2 Locality information for each seedlot screened for powdery mildew resistance (Continued).

Local #	Seedlot	Botanical Variety	Origin	Latitude	Longitude	Elevation (m)
1335	Rattlesnake Gulch	*neomexicanus*	Colorado, U.S.	39.931	-105.291	1890
1336	Eldorado Canyon S.P.	*neomexicanus*	Colorado, U.S.	39.930	-105.297	1890
1337	Eldorado Springs	*neomexicanus*	Colorado, U.S.	39.931	-105.282	1832
1338	Redstone #1	*neomexicanus*	Colorado, U.S.	40.515	-105.188	1722
1339	Redstone #2	*neomexicanus*	Colorado, U.S.	40.567	-105.230	1813
1341	Hayden East	*neomexicanus*	Colorado, U.S.	40.488	-107.159	1975
1347	Axial #3	*neomexicanus*	Colorado, U.S.	40.249	-107.785	2025
1359	Sweetwater #1	*neomexicanus*	Colorado, U.S.	39.810	-107.182	2366
1360	Sweetwater #2	*neomexicanus*	Colorado, U.S.	39.810	-107.171	2363
1361	Sweetwater #3	*neomexicanus*	Colorado, U.S.	39.798	-107.161	2363
1362	Silver Plume	*neomexicanus*	Colorado, U.S.	39.697	-105.724	2791
1363	Aspen	*neomexicanus*	Colorado, U.S.	39.185	-106.808	2437
1368	Cochetopa Creek	*neomexicanus*	Colorado, U.S.	38.458	-106.758	2439
1369	Tomichi Creek	*neomexicanus*	Colorado, U.S.	38.414	-106.512	2518
1373	Sangre de Cristo #2	*neomexicanus*	Colorado, U.S.	37.531	-105.295	2541
1377	Chimney Rock	*neomexicanus*	Colorado, U.S.	37.213	-107.298	2025
1379	Leopard Creek #2	*neomexicanus*	Colorado, U.S.	38.023	-108.055	2228
1364	Willow Creek	*neomexicanus*	Colorado, U.S.	38.453	-107.058	2357

Appendix 4.1. Diagrammatical representation of fungal growth of *P. macularis* on *H. l.* 'Symphony' at specific hours after inoculation.

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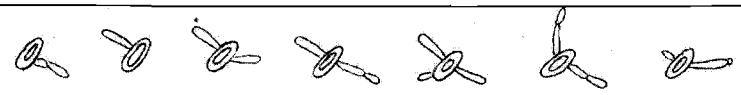
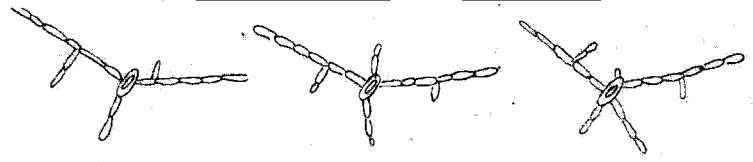
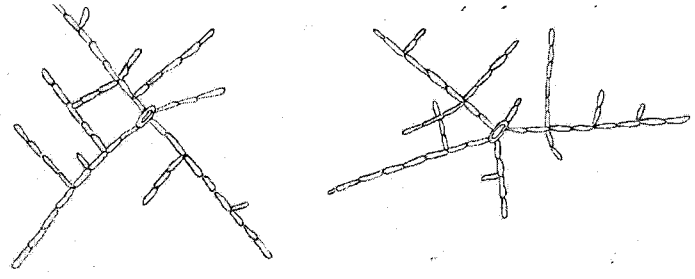
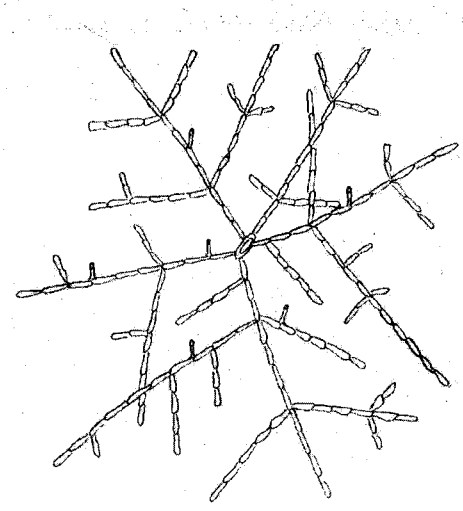
15 h	
24 h	
48 h	
72 h	

Appendix 4.1. Diagrammatical representation of fungal growth of *P. macularis* on *H. l.* 'Symphony' at specific hours after inoculation (continued).

96 h	
120 h