Initial Selection of *Humulus lupulus* L. for a Long-Term Breeding Program

Joshua Havill[1], Charlie Rohwer[2] and Vincent Fritz[2]

1. *Department of Horticultural Science, College of Food, Agriculture and Natural Resources, University of Minnesota, St. Paul, MN*
2. *University of Minnesota - Southern Research and Outreach Center, Waseca, MN*

Abstract

Selection of hops (*Humulus lupulus* L.), a dioecious perennial, in breeding programs has always been based on phenotypic evaluation of multiple genotypes. Evaluation of 239 progeny from 4 half-sibling families and 1 open-pollinated cross for powdery mildew (*Podosphaera macularis*) resistance, and quantification of pubescence and internode length resulted in selection of 150 progeny that will undergo further phenotypic analyses in field-grown conditions.

Introduction

Hops (*Humulus lupulus* L.) are a primary ingredient used in the brewing process for the production of beer. The majority of the world's hop production occurs in Europe and the Pacific Northwest (PNW) region of the United States. The total area harvested in the United States in 2012 was just below 32,000 acres (USDA 2012). The state of Washington alone produced 79 percent of hops grown in the U.S.

Cultivation of hops began approximately 1200 years ago in central Europe and has progressed quite rapidly in the past 100 years. Specific bitter resins are found in hops, primarily α- and β-acids, that are extracted during the brewing process. They are produced primarily within glands inside the inflorescences (strobili) of the plant, and contribute to the bitter flavors found in beer.

“Local” beers and breweries have contributed to the success of the craft beer industry. Various entrepreneurs have utilized the demand for local ingredients and are coordinating with growers to display the nuances of a fresh and local product (Moskowtiz-Grumdahl 2011), over what would have been normally imported from many other regions (PNW, New Zealand, and Europe).

Breeding programs have been developed in different countries to meet the needs of growers and brewers to develop new varieties with specific uses. The Midwest region of the United States has begun to see an increasing number of growers as the Midwest craft beer industries are continually expanded (Gilbert 2013). This expansion into Minnesota has led to the formation of the Minnesota Hop Grower’s Association which formed in March 2013 and currently communicates with over 75 growers (Foley, pers. comm.).

A key interest in development of breeding programs deals with communicating effectively with both brewers and growers to meet the needs of each. Local growers deal with photoperiods that differ between regions in Europe and the upper Midwest, while areas in the PNW are known for less summer rainfall and warmer springs. These differences alone contribute to determining which currently cultivated varieties might pose useful characteristics in Minnesota. Also of concern to growers are factors such as powdery mildew (*Podosphaera macularis*) and a foliar pest, the two-spotted spider mite (*Tetranychus urticae* Koch.). Presence of either within a production environment can result in detriment to the health of the plant, quality and yield (Neve 1991).

Male plants have little to no economic value and much has been done to eradicate them from regions where production occurs. Their usefulness in breeding programs is being realized as novel traits that are desirable for production may be introduced from wild hop plants.

Identifying young plants with novel traits is paramount to the success of any breeding program. Resistance to powdery mildew is of concern due to the rapid rate of reproduction and spread within a field, as well as persistence within the fields from overwintering inoculum. Complete crop losses are not unheard of but many factors play into the rate of incidence (Peetz et al. 2009). Making specific selections with resistance will increase capital gains of the grower and reduce the need for fungicides. Pubescence has been shown to impact resistance to mites in other species (Gillman et al. 1999), so data was collected from specimens to identify selections with interesting leaf hair traits that can be further analyzed for potential mite resistance.

The potential for low-trellis production is also of import for growers. The ability to permanently install a trellis system, with limited maintenance and upkeep, may increase safety (<10’ – 12’ vs. >16’), and easier scouting ability could lead to more precise pesticide use. A small number of dwarf or semi-dwarf varieties have been created (Summit™, First Gold, Pioneer, and Boadicea, for example), but they require substantial licensing fees to grow or are otherwise currently unavailable to growers in Minnesota. A common trait among these varieties is relatively short internode length. Observations of nodes per length on all plants described by this work displayed a heterogeneous population and could allow for selection of possible dwarf varieties.

Materials & Methods

Plant Material

Four crosses were made in 2012 with field-grown commercial cultivars ‘Chinook’, ‘Newport’, ‘Nugget’, ‘Spalter Select’. They were fertilized with pollen from a wild male plant, designated HRC-01M, which was collected from the University of Minnesota (UM) Horticultural Research Center (HRC) at the Minnesota Landscape Arboretum in Chanhassen, MN. One more cross resulted from an open-pollination event with a wild female plant (*H. lupulus* var. *pubescens*) germinated from seed collected by the USDA in Missouri, designated Rulo – E.1 (PI 617471).

Parent selection was based on varying characteristics. Rulo-E.1 is a pubescent variety which may result in selections with greater mite tolerance. Both ‘Spalter Select’ and ‘Newport’ are disease resistant and in theory would provide offspring with resistant traits. ‘Nugget’ bears some resistance and is vigorous and high-yielding. ‘Chinook’ is vigorous and has potential for dwarf traits (Rohwer, pers. comm.)

Of the 5 crosses made, 4 resulted in successful pollination of ‘Nugget’, ‘Chinook’, ‘Spalter Select’ and Rulo-E.1. Approximately 1,450 seeds were collected and were then stratified at 1.7° C starting 10 December through 29 January in a petri dish with filter paper wetted with 5 mg/L Captan and 0.3 percent PNO_3. Germinated seeds were planted in a soilless mix of Sunshine Mix #8 in 0.01L plug trays (288 cells). During March the seedlings were transplanted into 1204 inserts (0.1L cells, 48 cells) and the following month they were transplanted into 0.5 L pots. In late May/early June plants were again transplanted into a 3.5 L pot in a 3:1 soilless mixture of Sunshine Mix #8 (Sun - Gro Horticulture, Bellevue, WA) to Krum Horticultural Coarse Perlite (Silbrico Corporation, Hodgkins, IL). Plants were fertilized as necessary with 150 ppm 20-10-20 Peat – Lite Special water-soluble fertilizer (JR Peters, Allentown, PA) during this period. Seedling hops were grown at an average temperature of 22.1

– 25.8° C while maintained at the University of Minnesota Plant Growth Facilities (St. Paul, MN). In early July a portion of the plants were moved to the Southern Research and Outreach Center (SROC) in Waseca, MN and were again transplanted into 11.3 L pots with BFG Grower Select H2 growing media and given 80 grams Nutricote 13-13-13 Type 180 slow-release fertilizer (Arysta Life Science, Tokyo, Japan). The remaining plants were moved to the SROC in early August and then transplanted under the same conditions. All growing supplies were purchased from BFG Supply (Burton, OH).

Leaf Pubescence

Samples were collected from the youngest, fully expanded leaves of 82 plants growing in the greenhouse to quantify the density of pubescence from 28 May to 5 August. A 16 mm diameter cork-borer was used to puncture the leaf material and two sub-samples were collected from each cork-borer piece. The sub-samples measured 50.3 mm^2 and were then placed between a microscope slide and cover-slip underneath a SZH10 research stereomicroscope (Olympus Corporation, Center Valley, PA) at 50X magnification. Trichomes present on secondary and tertiary veins were counted separately from those present on the abaxial leaf surface and included in measurements as a part of total pubescence. The two sub-samples were shown to correlate with each other with respect to the total trichome density.

Internodal Distances

Measurements of internodal distances were collected on all plants growing in the greenhouse from 25 May to 5 August. Samples from each plant included individual measurements of two internodes consecutively along a stem. The average intermodal distance was calculated from the collected data and used to make determinations about potential for low-trellis production.

Powdery Mildew Scores

After having been moved to the field plots at SROC in late July, seedlings contracted powdery mildew from nearby plants in the field. The disease was allowed to spread thoroughly into the seedling population and was then scored based on phenotypic variance. Using modifications of the six – step ordinal system developed by Henning et. al (2011), a five – step ordinal system was created. After 14 days of incubation, plants were scored 0 – 4, where 0 = no symptoms of infection (resistant); 1 = blisters, necrotic flecks or spotting (moderately resistant); 2 = few lesions present on a small number of leaves (tolerant); 3 = multiple lesions present on few leaves or few lesions on many leaves with coalescent colonies (susceptible); 4 = multiple coalescing lesions on all susceptible leaves (highly susceptible).

Results and Discussion

Phenotypic ratings for powdery mildew represent a high variance between crosses (Fig. 1). Individuals categorized as "resistant" (0 – 2) totaled 28.9 percent of the entire population, while the remaining 71.1 percent were placed into the "susceptible" (3 – 4) group. The "resistant" group had a mean score of 1.88 and the "susceptible" group had a mean score of 3.27.

Inheritance of the gene conferring resistance in *H. lupulus* follows a single gene model under cultivated varieties and a polygenic model when wild hops are used. The implementation of using major resistance genes in combination with partial resistance may prove most useful and more durable than either one alone (Darby et al. 1989, Darby 2001, Godwin et al. 1987, Smith 2005).

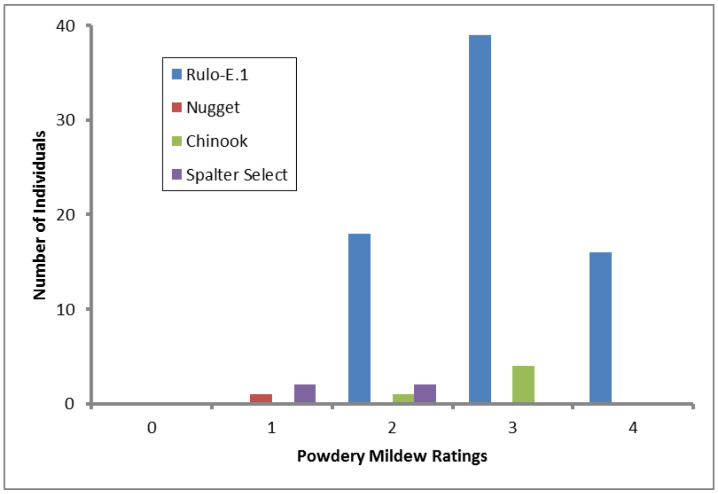

Fig. 1 Frequency histogram of powdery mildew ratings for individuals resulting from 3 half-sibling families (Seedlings from 'Nugget' x HRC-01M, 'Chinook' x HRC-01M and 'Spalter Select' x HRC-01M) and 1 open-pollination event (Rulo-E.1 x OP) of *Humulus lupulus* L. Ratings based on a five-step ordinal scale (0 = resistant, 4 = highly susceptible).

Seedlings from Rulo-E.1 x OP exhibited a wide range of trichome densities as well as internodal distances (Fig. 2) while crosses of HRC-01M x ('Nugget', 'Chinook' and 'Spalter Select') displayed slight differences in internodal distances and much less variance within each population than Rulo-E.1. Select seedlings from all 4 crosses exhibit qualities that are desirable for low-trellis production (dwarf traits) and would also be useful in determining the impact trichome density would have on resistance to two-spotted spider mite (*Tetranychus urticae* Koch.)

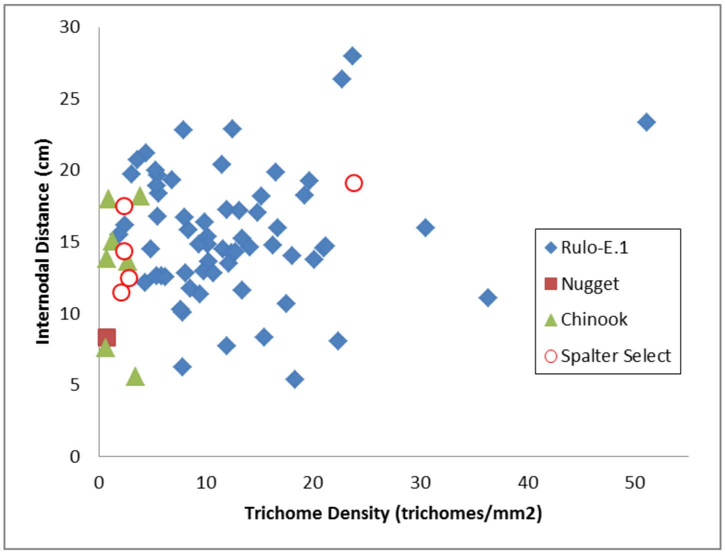

Fig. 2 Maternal effects on inheritance of trichome density and internode length for individuals resulting from 3 half-sibling families (Seedlings from 'Nugget' x HRC-01M, 'Chinook' x HRC-01M and 'Spalter Select' x HRC-01M) and 1 open-pollination event (Rulo-E.1 x OP) of *Humulus lupulus* L.

Selections

Selections were performed based on all evaluated phenotypic data. Plants that had combinations of low (<9 hairs/mm^2) or high (>25hairs/mm^2) trichome density and short (<14 cm) internodal distances were kept for further studies. Of the 239 plants that were germinated and grown, 150 were placed into the field based upon the observed data.

Further analysis of collected data provides evidence that phenotypic selection based on petiole trichome density when performed initially on large populations might be a useful indicator of total pubescent characteristics. A significant positive correlation was found between petiole trichome and leaf trichome densities ($\rho = 0.52$, results not shown). Similar correlations have been identified in other agriculturally important crops (Hornbeck and Bourland 2007) and further emphasis in trait selection might benefit resistance characteristics (Glas et al. 2012).

Future studies on these populations will include estimation of vigor in a field-production setting, determination of sex for breeding purposes, further analysis of disease resistance, and extraction and characterization of α-acid and xanthohumol content. Xanthohumol has been implicated in specific human health benefits that are associated with other prenylated and phenolic compounds (Magalhàes et al. 2008). Studies will also be performed with field-grown material to determine the impact pubescence has on mite resistance.

Acknowledgements

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