

Integrating Molecular Tools and Conventional Approaches in the Oregon State University Hazelnut Breeding Program

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The Oregon State University (OSU) hazelnut breeding program, initiated in 1969, continues to develop new cultivars for the hazelnut industry that combine suitability to the blanched kernel market with resistance to eastern filbert blight (EFB) caused by *Anisogramma anomala*. Oregon's hazelnut growers support the program through the Oregon Hazelnut Commission. The need for improved disease-resistant cultivars and pollinizers is the program's driving force. About 5000 seedlings have been planted each year during the past 2 decades, and 10 cultivars and 10 pollinizers have been released. Oregon produces 99 percent of the United States crop, but only 3 to 5 percent of the of the world crop. Beginning in 2009, plantings have expanded from 12,000 ha by 1200 ha per year. Most new orchards are being planted to 'Jefferson,' increased by micropropagation.

Cultivars of European hazelnut (*Corylus avellana* L.) are highly heterozygous and clonally propagated. Hazelnuts were already being cultivated in Italy and Spain at the time of the Roman Empire as well as at an earlier date on the Black Sea coast of Turkey and in the Caucasus Mountains. All cultivars in these countries were selected from the local vegetation. *Corylus avellana* is native to most of Europe, Turkey, and the Caucasus republics where it grows as a small-stature, multi-stemmed shrub. Diverse germplasm has been imported as scions or seeds. The Corvallis collections contain ~900 accessions and include representatives of all 11 *Corylus* species and ~500 clones of *Corylus avellana*. Hazelnut is a model for the order Fagales and family Betulaceae. All *Corylus* species are diploid, have a small genome (0.48 pg/1C, 380 MB), and an estimated 35,000 genes. Morphology, phenology, and all marker types investigated to date have shown *C. avellana* to be highly polymorphic.

Objectives of the OSU hazelnut breeding program fall into two categories: suitability to the blanched kernel market and resistance to EFB. Some 93 percent of the world's hazelnuts are sold as kernels to chocolate makers and bakers; only 7 percent are sold in-shell for the table market. Kernel varieties should be resistant to bud mites (primarily *Phytoptus avellanae*), start to bear nuts at an early age, have consistent high nut yields, round nut shape, thin shells, easy pellicle removal, few nut and kernel defects, early nut maturity, and free-falling nuts. The process to develop varieties begins with 40 to 50 controlled pollinations each year. The hybrid seeds are harvested in late August and are soaked and placed in stratification in early November. The seedlings are grown in 4 L pots in the greenhouse in the first year and then transplanted 1 m apart in the field in October in rows that are 3 m apart. Most seedlings bear their first nuts in the fifth leaf. Evaluation of the seedlings in years 5 to 8 reduces their number to 25 to 40 selections, which are propagated by tie-off layerage. The layers are lined out in a nursery row for one season and then used to plant replicated yield trials. Data is collected from the trials in years 3 to 7. The breeding cycle from seed to seed is 8 years. New cultivars are released 16 to 17 years after the cross is made.

A few traits are controlled by single loci, including pollen-stigma incompatibility and some EFB resistance sources. Most of the program's breeding objectives involve quantitative traits for which heritability was estimated by regression (Mehlenbacher et al. 1993, Yao and Mehlenbacher 2000). Heritability is >50 percent for most traits we have investigated, so it is unlikely that marker-assisted selection will be more effective than phenotypic selection.

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Greenhouse inoculation with EFB is used to classify accessions as resistant or susceptible. Three trees per accession are grown in the greenhouse, inoculated with a spore suspension, and maintained under high humidity. The trees are placed outside where they go dormant, receive chilling, and then develop cankers 15 to 20 months after inoculation. Very high resistance has been identified in 23 accessions, which represent about 2 percent of those tested (Chen et al. 2007, Lunde et al. 2000, Sathuvalli et al. 2010b). Several resistant accessions have been used as parents in breeding. The resistance in 'Gasaway' is conferred by a dominant allele at a single locus which is carried by the recently released 'Yamhill' and 'Jefferson.' A very high level of resistance was detected in accessions imported as scions: the Spanish 'Ratoli' and 'Culpla,' Georgian OSU 759.010, Serbian 'Crvenje' and 'Uebov,' and eight selections from a forestry institute near Moscow, Russia. Seeds have been imported from several sources, and selected trees in these populations have been tested for EFB response. Highly resistant selections include OSU 408.040 from Minnesota; OSU 495.072 from Russia; and selections from southern Russia, Crimea (Ukraine), and the Republic of Georgia. DNA marker-assisted selection (MAS) has been practiced for 'Gasaway' resistance for more than 12 years. The MAS was initiated because of the pathogen's 2-year life cycle, a desire to select for resistance in the absence of the pathogen, and difficulties in determining disease response. Random Amplified Polymorphic DNA (RAPD) markers 152-800 and 268-580 flank the 'Gasaway' resistance locus, are robust and easy to score, and are useful in multiple populations (Mehlenbacher et al. 2004). Seedlings that carry one or both RAPD markers are planted in the field. Resistance from 'Ratoli,' OSU 408.040, and Georgian OSU 759.010 is also simply inherited, and linked DNA markers have been identified (Chen et al. 2005; Sathuvalli et al. 2010a, 2011).

A linkage map for hazelnut was constructed using RAPD markers (Mehlenbacher et al. 2006), and microsatellite markers were added to serve as anchors. EFB resistance from 'Gasaway' was assigned to linkage group 6 (LG6), from 'Ratoli' to LG 7, and from Georgian OSU 759.010 to LG 2. We will use DNA markers to pyramid different resistance genes. Microsatellite markers show transferability across genera in the Betulaceae (Gürcan and Mehlenbacher 2010), and have been used to fingerprint accessions and study genetic diversity.

Susceptible cultivars vary widely in level of susceptibility. To quantify susceptibility, cultivars and selections are propagated by tie-off layerage. The rooted layers are grown for 1 season in pots in the lathhouse. In the spring, 12 potted trees of each cultivar (including checks) are placed under a structure topped with diseased wood. Sprinklers keep the cankered branches wet, and spores rain down on the young shoot tips. After several weeks of exposure, the trees are planted in a nursery row where cankers are allowed to develop. Cankers are counted and measured 18 to 20 months after exposure. The total length of cankers is calculated for each tree, and a square root transformation used to reduce the relationship between mean and variance. Quantitative resistance, expressed as fewer and smaller cankers, is present in OSU releases 'Lewis,' 'Clark,' and 'Sacajawea'. In a set of nine progenies, the heritability estimate for proportion of wood diseased was 47 percent (Osterbauer et al. 1997).

Oregon State University has partnered with Rutgers University, the University of Nebraska-Lincoln, and the Arbor Day Foundation to form the Hybrid Hazelnut Research Consortium. The Consortium's goal is to develop *C. americana* × *C. avellana* hybrids with EFB resistance and adaptation to the climate of the eastern and Great Plains states. The high disease pressure in field plots at Rutgers University provides useful information.

With Todd Mockler and Illumina, the genome and transcriptome of 'Jefferson' hazelnut was sequenced, and seven accessions are being resequenced. The assembled sequences are being mined for microsatellites and single nucleotide polymorphisms (SNPs), with a special interest in markers for newly identified EFB resistance genes. Map-based cloning of the 'Gasaway' EFB resistance gene identified five candidate genes. A similar approach is in progress for the incompatibility locus. For use in marker-assisted selection with 4000 seedlings per year, markers must be high throughput, robust, and valid in different populations. For use with 400 selections per year, other marker types such as microsatellites are feasible. SNPs are abundant in the hazelnut genome (~1 in 50 bp). High

Resolution Melting (HRM) analysis using a LightScanner (Idaho Technology) is promising for cost-effective scoring of SNP markers. However, MAS is less useful for traits with high heritability, and most traits in hazelnut are highly heritable.

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