Hazelnut Consortium Update: Eastern North American Progress Against Eastern Filbert Blight

Thomas J. Molnar, Ph.D.
Plant Biology and Pathology Dept.
Rutgers University

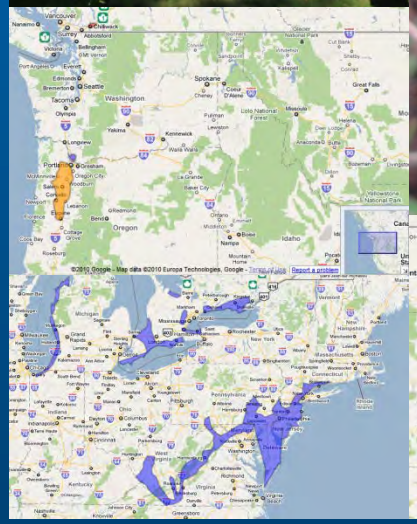
4th Annual Hazelnut Symposium
Brantford, Ontario
March 19, 2013

Hazelnut Consortium Partners:

- Oregon State
  - Shawn Mehlenbacher, Vidyasagar Sathuvalli, Dave Smith
  - Hazelnut breeding and genetics, *Corylus* molecular biology
- University of Nebraska/Nebraska Forest Service
  - Scott Josiah, Troy Pabst, Milford Hanna, Loren Isom
  - Replicated field trials, outreach, oil research, new product development
- National Arbor Day Foundation
  - Doug Farrar, Adam Howard
  - Outreach/disseminate information, Organization/ Management
- Rutgers University
  - Tom Molnar, Brad Hillman, Guohong Cai, John Capik
  - Hazelnut breeding and genetics, EFB molecular biology

Overriding Goal of the Hazelnut Consortium

- This collaboration leverages substantial resources and prior investments to address the major factors that threaten the sustainability of current hazelnut production in Oregon and severely restricts expansion in North America:
  - susceptibility to eastern filbert blight (EFB) caused by *Anisogramma anomala*
  - the limited climatic adaptation of existing cultivars



Short-term goals:

- Develop genomic tools for the hazelnut and EFB pathogen, and use them to:
  - supplement traditional breeding practices
  - study genetic variation in both organisms
  - improve our knowledge of host-pathogen interactions to develop plants that express more durable forms of resistance
- Continued collection of *Corylus* genetic resources with an emphasis on native *C. americana* and cold-hardy *C. avellana* from northern Europe
  - Genetic variability exists to address both limiting factors (EFB and climate)
- Collaboratively develop improved EFB-resistant hazelnut selections (*C. avellana* and hybrids)
- Explore alternative hazelnut-related products
- Establish clonal replicated yield trials of new hazelnut selections with:
  - Rutgers and University of Nebraska, Lincoln
  - other university cooperators
  - potential growers

Some specific projects:

- SSR (microsatellite) marker assessment of diversity in *C. avellana*, *C. americana,* & hybrids
- Map-based cloning: 'Gasaway' gene is complete– next target is the self-incompatibility system
- Sequence the European hazelnut genome to develop genetic tools for breeding (marker assisted selection, etc.)
- Sequence EFB pathogen genome, develop markers to study population biology and diversity
- Develop Real-time PCR detection assay for EFB pathogen (rapid screening test)
- Collect and evaluate diverse *C. americana* germplasm; develop/evaluate new hybrids
- Assess kernel oil content, kernel composition, and other attributes

A preface to our collaborative work:
Hazelnuts in eastern North America

- Early colonists brought hazelnuts from Europe–very few records, no production established
- The fungal disease **Eastern Filbert Blight** (EFB) killed most European hazelnut (*Corylus avellana*) trees
- Disease is occurs naturally on the wild American hazelnut, *C. americana*
- EFB is the **primary reason** no commercial hazelnuts are grown in the east

Native range of wild American hazelnut and associated pathogen *Anisogramma anomala* that causes Eastern filbert blight (EFB)

Corylus americana

- While the native hazelnut is cold hardy and tolerant of EFB, its nuts are tiny and thick shelled
- Plus, nuts do not drop from the husk and some remain attached to branches after maturity

Wild *Corylus americana* in New Jersey

European hazelnut (*Corylus avellana*)

At Corvallis, Oregon USDA Repository

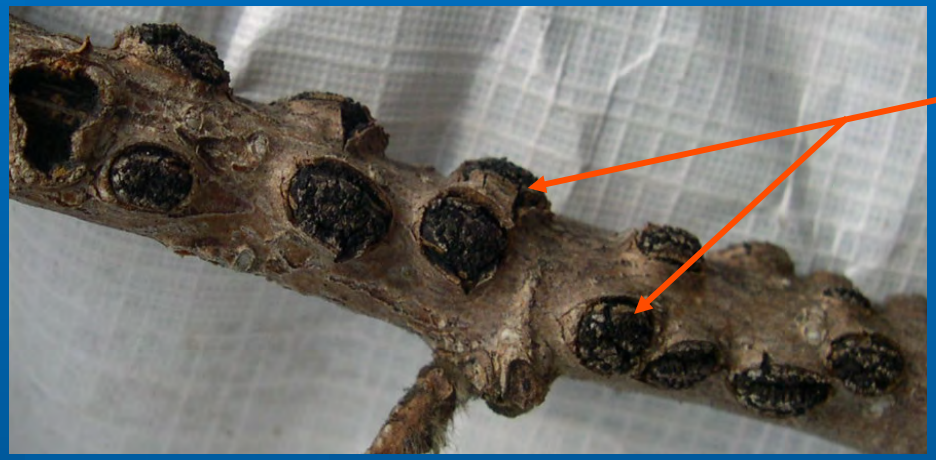
- 825 accessions of *Corylus*
- 429 of *C. avellana*
- ~ 100 more at OSU

www.plantyfolia.com/photos106/corylus_ens.jpg

http://caliban.mpiz-koeln.mpg.de/~stueber/thome/band2/tafel_005.jpg

www.funghiitaliani.it/Alberi/nocciolo/Corylus%2520avellana1.jpg

Eastern Filbert Blight
Fungus - *Anisogramma anomala*



Stromata
(fruiting body)

- Wild hazelnut (*C. americana*) harbors EFB across its wide native range – it provides the initial inoculum source
- Most European hazelnuts are highly susceptible
- Spreads during periods of rain in the spring
- Fungus grows under bark and, when reproducing, creates cankers that kill the trees

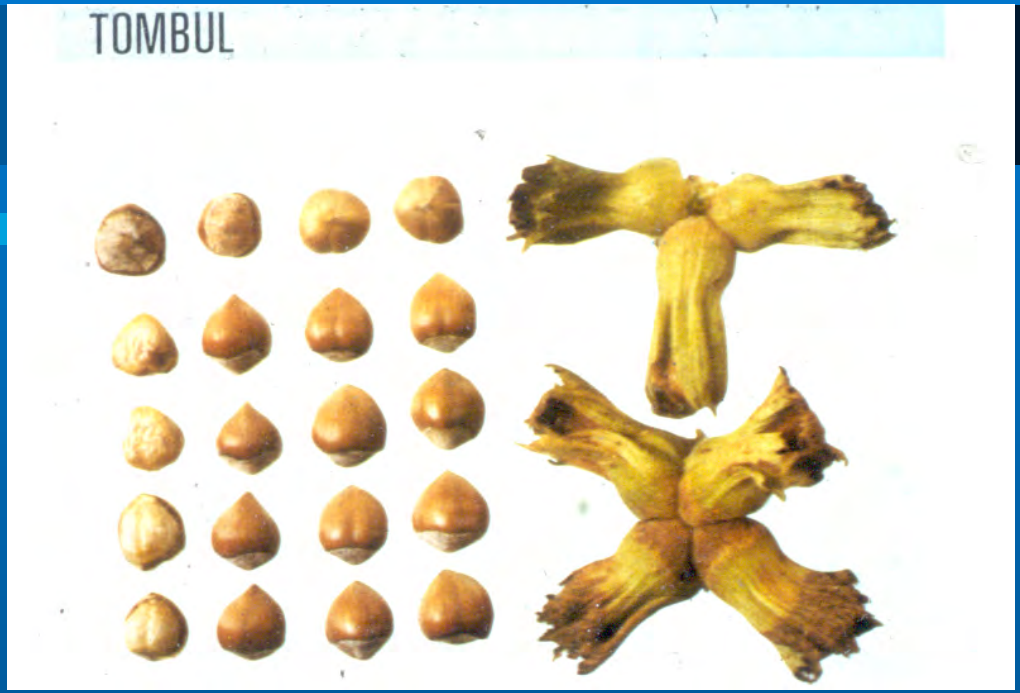
Anisogramma anomala

- Ascomycete in the order Diaporthales
  - same order as chestnut blight and dogwood anthracnose pathogens
- Obligate biotroph of only *Corylus*
  - infects only living hazelnut tissue
- Found only in North America
  - would be quite devastating in Europe where *C. avellana* is a common understory tree


5-year-old C. avellana seedlings in New Jersey

Hazelnut Production (MT)

1.	Turkey	504,000	71.2%
2.	Italy	116,500	16.4%
3.	Azerbaijan (est.)	35,000	4.9%
4.	United States	27,000	3.8%
5.	Georgia (est.)	25,000	3.5%
6.	Spain	18,000	2.5%



Hazelnuts in Turkey are grown on the steep slopes of the Black Sea coast

Tombul is an important cultivar.

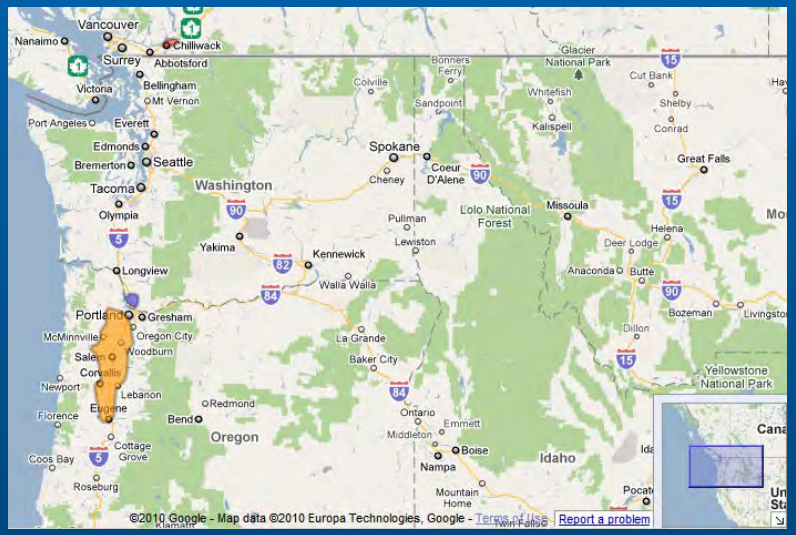
It has long, clasping husks and

small nuts for the kernel market.

(photos: Shawn Mehlenbacher)

Hazelnuts in the Pacific Northwest

- Hazelnuts were first brought to the Pacific Northwest in the late 1800s
- European cultivars were well-adapted to the coastal valleys of Oregon and Washington and **no eastern filbert blight was found there**
- The Hazelnut industry thrived for nearly 100 years



99% of U.S.A production is in the Willamette Valley of Oregon

Hazelnuts in Oregon are mechanically harvested.

Nuts of ‘Barcelona’ fall free of the husk at maturity. The large nuts are suited to the in-shell market.

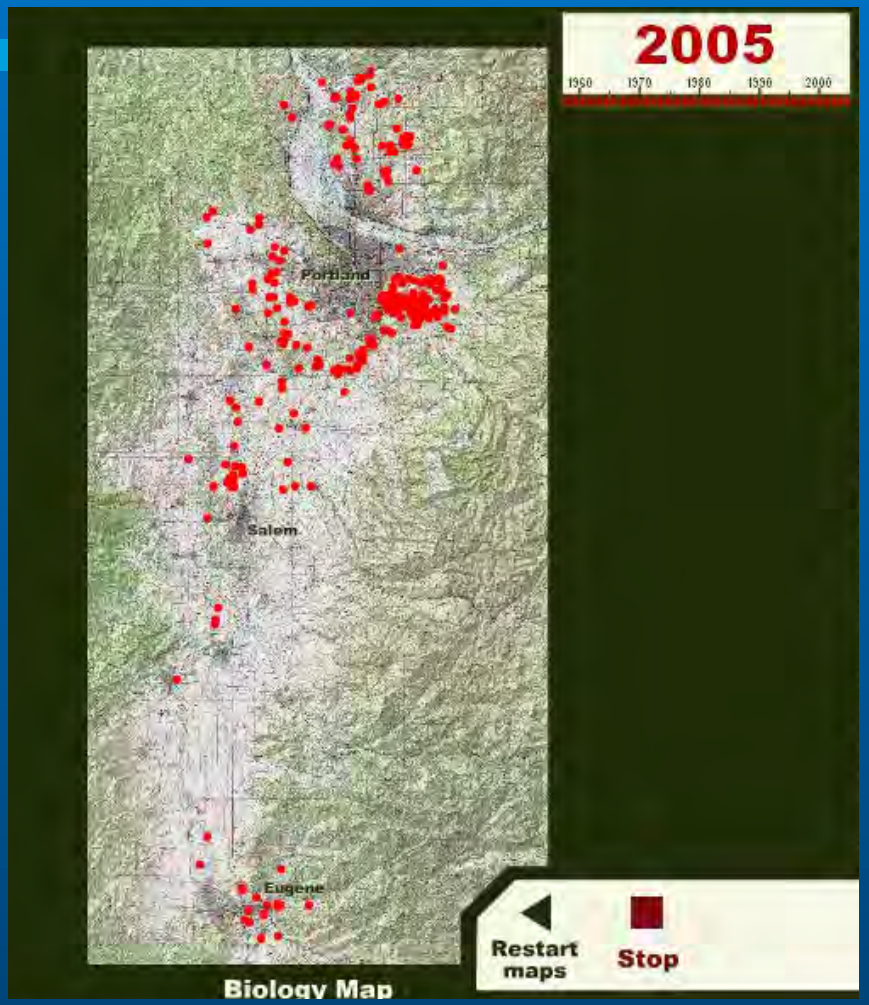
(photos: Shawn Mehlenbacher)

FIGURE 3. Natural distribution of *Corylus avellana* L.

The distribution of *Corylus avellana* includes many climatic zones, but commercial production regions are limited (Mehlenbacher, 2003)

EFB invades Washington State

- EFB was found in southwest Washington in the 1960s leading to a disease epidemic and major orchard losses in Washington and later Oregon
- Fortunately, prevailing weather patterns slowed its spread southward into the Willamette Valley where a majority of the production orchards are located
- In the 1970s, research on the fungus was initiated at Oregon State University



http://oregonstate.edu/dept/botany/epp/EFB/location/map1.htm

Oregon State University (OSU)

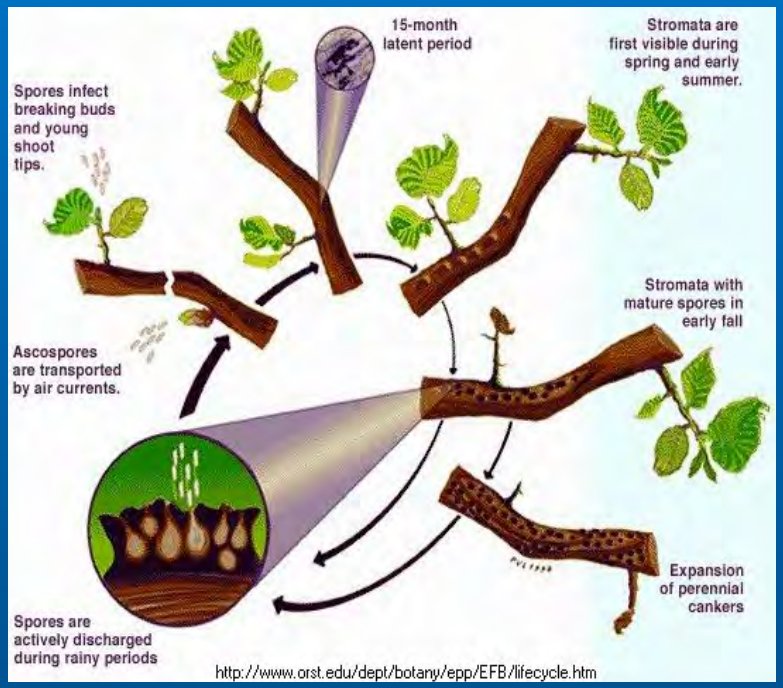
- OSU has the largest hazelnut research and breeding program in the world
  - Currently lead by Shawn Mehlenbacher (previously Maxine Thompson and Harry Lagerstedt)
  - Breeding program started 1969
  - Focused on kernel market 1979
- It was the only public hazelnut breeding program in the U.S. for many decades
- OSU and the U.S. Department of Agriculture, Agriculture Research Service, National Clonal Germplasm Repository hold over 800 hazelnut accessions
  - Includes representation of all the major *Corylus* species
  - New accessions added each year

Oregon State UNIVERSITY

OSU and the USDA germplasm repository are located in Corvallis, Oregon. The climate is mild—very similar to the Mediterranean region

Eastern Filbert Blight Research

- Today, we have a much greater understanding of the biology and lifecycle of EFB and control measures have been developed
- Control includes disease scouting, pruning, and copious applications of fungicides
- However, hazelnuts are traditionally a low-input crop
  - the development of resistant cultivars is the only sustainable means for control
- The search for resistance to EFB in European hazelnut began at OSU in the 1970s, and included methods to better identify resistant plants



Lifecycle of *Anisogramma anomala*
http://oregonstate.edu/dept/botany/epp/EFB/

Breeding EFB resistant plants at OSU

- 'Gasaway' (an old, late-shedding pollinizer with small nuts) was the first cultivar identified with resistance to EFB
  - Found to transmit a dominant gene for resistance
  - Widely used in OSU breeding program
- EFB-resistant cultivars carrying the 'Gasaway' gene were released in Oregon starting in 2005
  - 'Santiam', 'Yamhill', and 'Jefferson'
  - 'Dorris' and 'Wepster' in 2012 and 2013, respectively
- New EFB-resistant cultivars are revitalizing the Oregon industry
  - Tree sales by OR nurseries indicate plantings of 1200 ha per year for the past three years (primarily 'Jefferson')

'Yamhill' small size for the kernel market

'Jefferson' large nut replacement for 'Barcelona'

(photos: Shawn Mehlenbacher)

Today, many resistant *C. avellana* have been identified in OR and are being used in breeding

Genotype	Origin
'Gasaway'	Washington
'Zimmerman'	Oregon
OSU 408.040	Minnesota "Weschcke Sdlg"
'Ratoli'	Spain
Georgian 759.010	Republic of Georgia
OSU 495.072	Russia
COR 157	Finland
'Culpla'	Spain
'Medium Long'	Geneva, NY, USA

Resistant *Corylus avellana* list continued…

Genotype	Origin
'Crvejne'	Cacak, Serbia
'Uebov'	Cacak, Serbia
Moscow Selections (5)	Russia
OSU 1187.101	Russia (Holmskij)
Seedlings (2)	Russia
Seedlings (3)	Crimea, Ukraine
Seedlings (2)	Republic of Georgia

(~2% of accessions show very high resistance)

Very high resistance to EFB in other *Corylus* species

C. americana 'Rush', now in BC_2 generation

C. americana 'Winkler', now in BC_1 generation

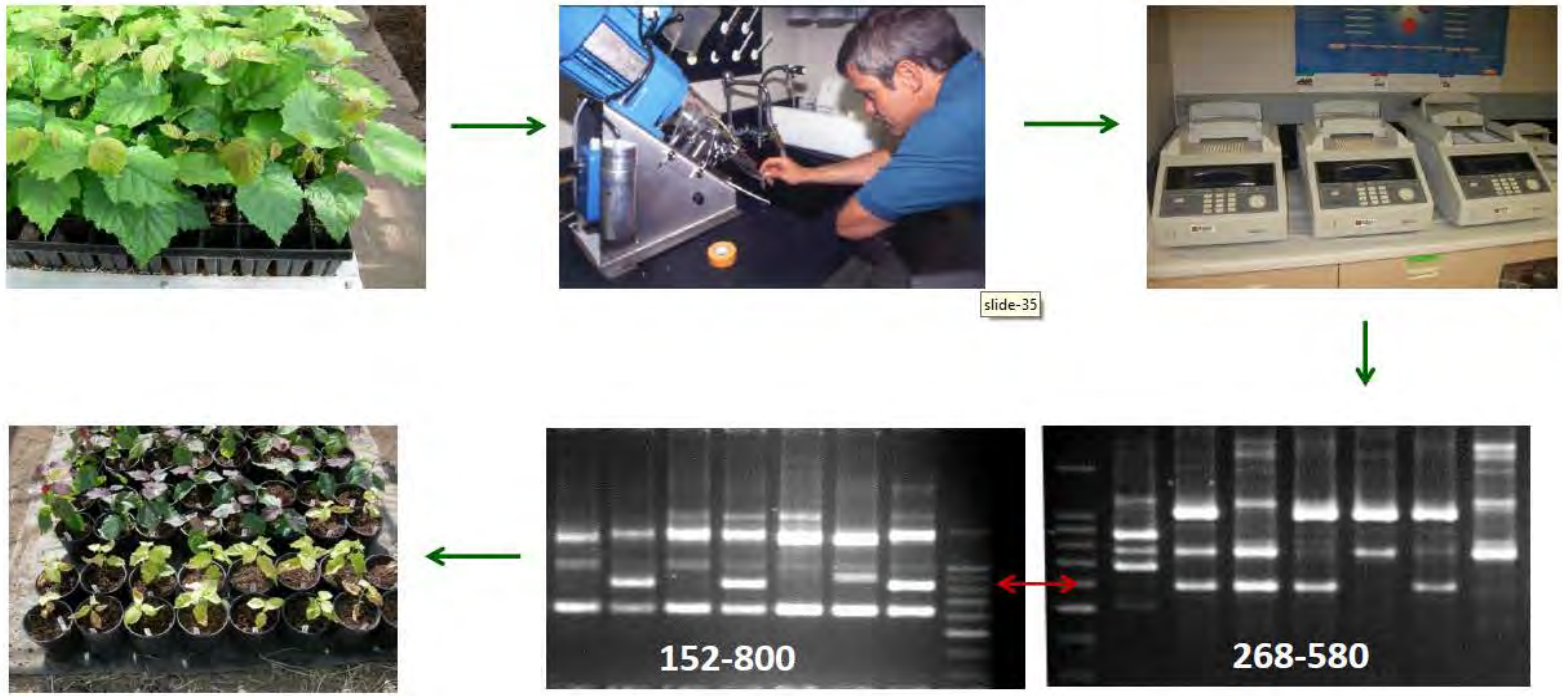
C. heterophylla 'Ogyoo', now in BC_2 generation

Segregation ratios indicate single loci with dominant resistance (discussed in more detail this afternoon)

Single genes are easier to work with in breeding, but might not be durable in the long term

OSU Next steps: molecular markers linked to EFB resistance to expedite breeding for resistance

- Extract DNA from ~ 3000 seedlings per year, 192 per day
- Amplify using flanking RAPD markers 152-800 and 268-580
- Seedlings that lack markers are discarded

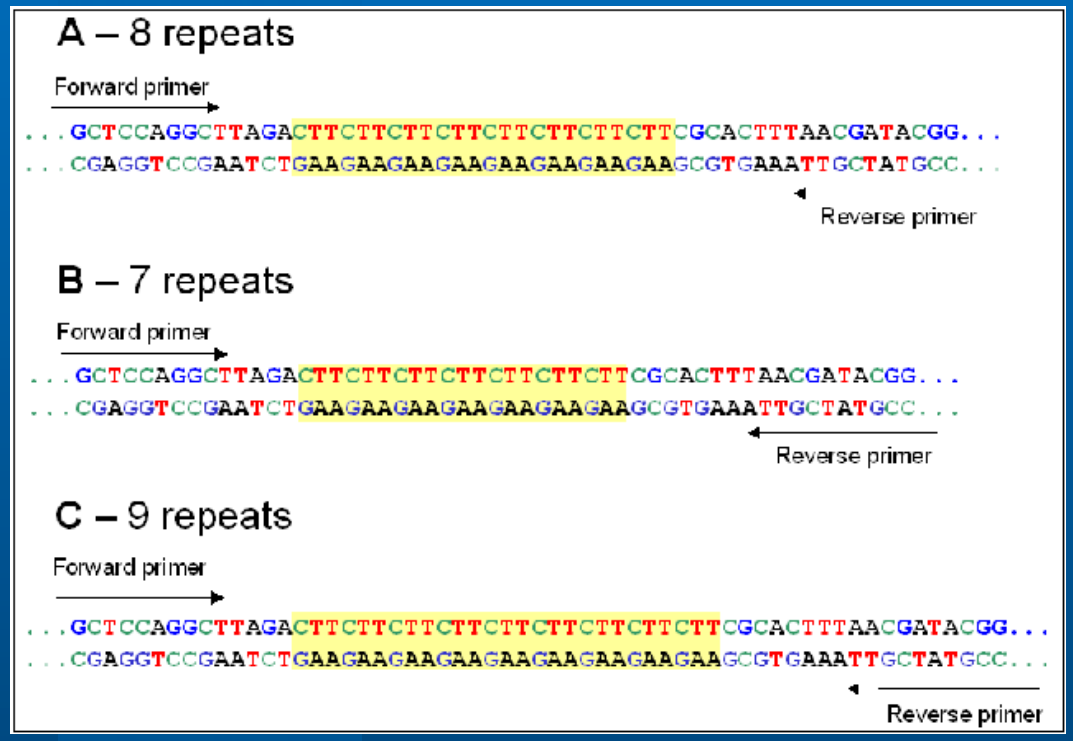


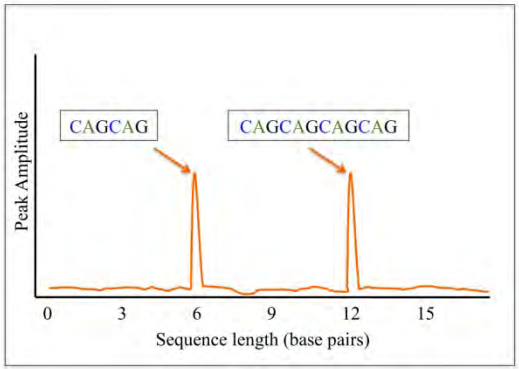
(photos: Shawn Mehlenbacher and Vidysagar Sathuvalli)

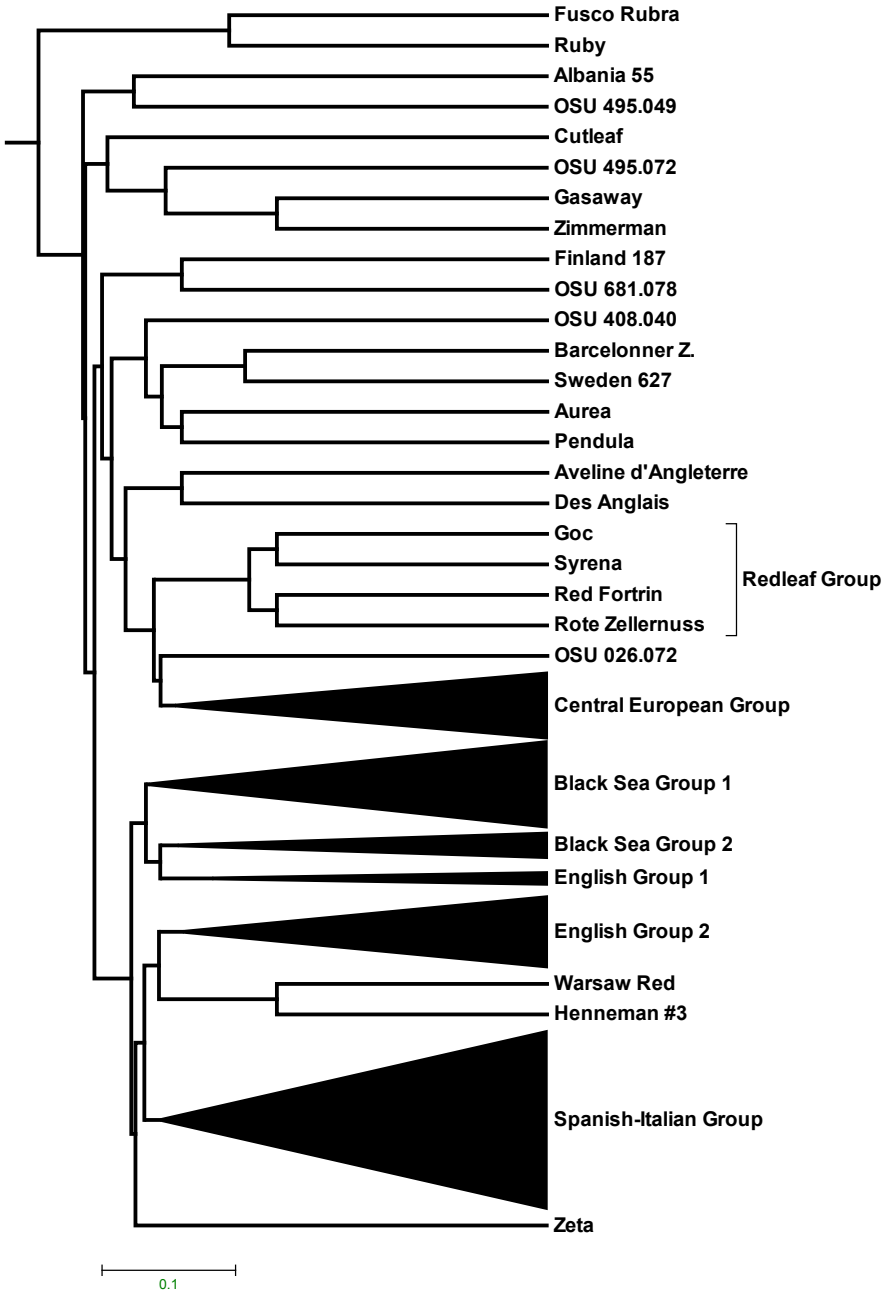
OSU, USDA, and Italian scientists developed hundreds of Simple Sequence Repeat (SSR) markers for hazelnut – useful for fingerprinting studies and genetic mapping

- Simple sequence repeats (SSRs), or microsatellites, are repeating sequences of base pairs of DNA
- SSRs are highly conserved within a species and often display polymorphisms
  - Specific gene regions found in all genotypes of a species, but the number of repeats differ for each genotype
  - Looking at differences between SSRs allows us to reveal genetic variability
  - Related plants share similar patterns of repeats (collectively represent the “Fingerprint” of the genotype)
- Using available technology, including genome sequencing, you can identify 1000s of SSRs for a given species

SSRs illustrated







Genetic diversity in *Corylus avellana* based on SSR markers

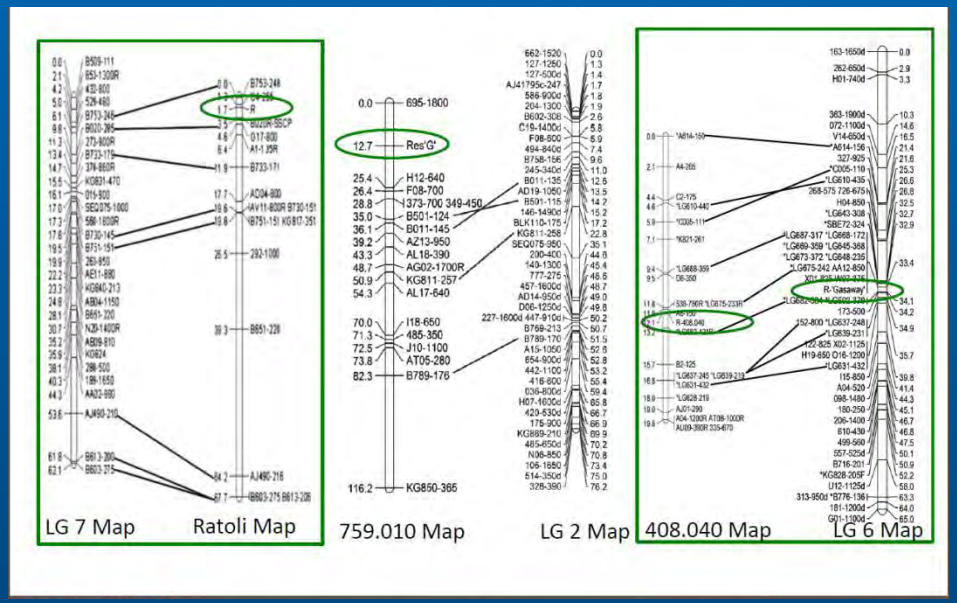
- Dendrogram based on microsatellite marker data shows four main groups:
  - Central European
  - Black Sea
  - English
  - Spanish-Italian
- 270 accessions incl. 72 synonyms
- (Gökirmak et al., 2009)
- Parentage is indicated for 31 accessions

Assignment of EFB Resistance Loci to Linkage Groups

(based on co-segregation with SSR markers)

In other words – what chromosome is the *R*-gene located on? This is the first step to finding out if they are different genes. Can you pyramid multiple genes in a single plant?

Gasaway	LG 6
Ratoli	LG 7
Georgian OSU 759.010	LG 2
OSU 408.040	LG 6
Culpla	LG 6
Russian OSU 495.072	LG 6



(photos: Shawn Mehlenbacher and Vidysagar Sathuvalli)

The European hazelnut genome has been sequenced

- OSU has sequenced the genome of 'Jefferson' using Illumina technology, resulting in 46.1 Gb of sequence data equivalent to 115X coverage of the genome
  - Illumina platform generates thousands of 150-250 base pair (bp) fragments that need to be organized into larger, contiguous pieces
    - From these smaller fragments, the programs Velvet and MIRA assembled 333,492 contigs with an average length of 1354 bp
    - Thus the genome sequence is many small sequences rather than 11 pairs of chromosomes
- The entire genome of *C. avellana* ('Jefferson') is now available for research http://hazelnut.cgrb.oregonstate.edu/
  - Additional work is underway to assemble the fragments, identify genes, etc.

How to use the sequence info?

- Improve understanding (disease resistance, incompatibility)
- Knowledge of genetic diversity, manage collection, choose parents
- Choose seedlings based on marker-trait associations
  - Reduce 4000 seedlings/yr to field vs. 400 selections/yr
- For seedlings, markers must be high-throughput and robust
- Single nucleotide polymorphisms (SNPs) are abundant in plant genomes
  - SNPs are even better than SSRs for fingerprinting and mapping genes

Univ. of Nebraska, Lincoln/ Nebraska Forest Service

- Field trials to evaluate available cultivars in Nebraska
- Working to identify high yielding hybrid selections of *C. avellana* x *C. americana*
  - Several consistently high yielding plants identified from Arbor Day Farm planting
  - 10 selections evaluated at Rutgers for EFB resistance with ~5 showing no disease after 8 years
- Testing seedlings and clones from OSU and Rutgers for adaptation to Nebraska
- Examining the potential of hazelnuts for markets other than food

The University of Nebraska farms provide stressful climate compared to NJ and OR: very cold through very hot, windy and dry

Hybrid Hazelnut Research at the Univ. of Nebraska

Category	Properties
Physical Characteristics	**Nut yield** **Nut/kernel size and shape** **Kernel percentage**
Oil Quality for Food Application	**Fatty acid profile** **Tocoherol and phytosterol** **Oxidative stability**
Oil Quality for Biodiesel	**Oxidative onset temperature** **Cloud point** **Kinematic viscosity** **Heat of combustion** **Free fatty acid**
Meal Quality for Food and Feed Applications	**Proximate analyses** **Mineral compositions** **Amino acid profile** **Anti-nutrients**

Hazelnut research at UNL

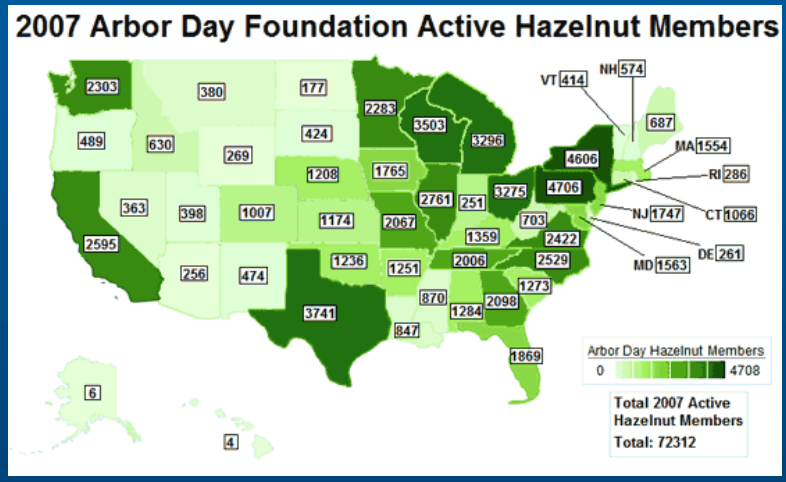
- Xu et al. 2012 Nutritional composition and **antioxidant activity in shells** from US-grown cultivars. Int. Journal of food Science and Technology. 47:940-946
- Xu et al. 2011. Evaluation of **Mold and mycotoxin contaminations** in hybrid hazelnuts grown in Nebraska. Journal of Food processing and Technology.
- Xu and Hanna. 2011. Nutritional and anti-nutritional compositions of **de-fatted Nebraska hybrid hazelnut meal**. International Journal of Food Science and Technologu. 46:2022-2029
- Xu and Hanna. 2010. **Composition and oxidative stabilities of oil** extracted from hybrid hazelnut grown in Nebraska, USA. Int. Journal of Food Science and Technology. 45:2329-2336

Hazelnut research at UNL

- Xu and Hanna. 2010. Evaluation of Nebraska hybrid hazelnuts: nut/kernel characteristics, kernel proximate compositions, and **oil and protein properties**. Industrial crops and Products. 31:84-89
- Xu and Hanna. 2009. Synthesis and characterization of **hazelnut oil-based biodiesel**. Industrial Crops and Products. 29:473-479
- Xu et al., 2009. Hybrid hazelnut **oil characteristics** and its potential oleochemical application. Industrial Crops and Products. 26:69-76

Arbor Day Foundation™

- Began their hazelnut research project in 1996 with plantings of hybrid hazelnuts at Arbor Day Farm in Nebraska City, Nebraska
  - *C. avellana* x *C. americana* from Badgersett (5,000 sdlgs)
- In 2000, the project evolved to include charter members across the nation growing hazelnuts in their backyards and reporting results back to the Foundation
- In 2010, the program reached 100,000 active members
- Responsible for outreach, coordinating consortium activities, and hosting website
  - http://www.arborday.org/programs/hazelnuts/consortium/



Arbor Day Foundation™

- Over 1 million members get the quarterly Arbor Day Newsletter
- Many thousands of visitors each to Arbor Day Lodge get the opportunity to walk in the large hazelnut orchard adjacent to the building
  - Host many important national meetings
- Spring issue is focused on hazelnuts
- Provides a lot of national attention for hazelnuts, when combined with all of the recent growth in demand and advertising for hazelnut products, awareness and interest in the crop appears to be growing considerably

Rutgers University

- Nut tree project started by successful turfgrass breeder Dr. C. Reed Funk
  - we have been researching nut trees at Rutgers University since 1996
- Original title of the project: Underutilized Perennial Food Crops Genetic Improvement Program
- Today, our main focus is hazelnut
  - Studying the EFB-pathogen
  - Identifying sources of resistance
  - Developing improved, EFB-resistant plants for New Jersey (northeast NA)

Tom Molnar and Reed Funk
Rutgers Adelphia Research Farm
August 2001

Rutgers University: Major role in Consortium is studying EFB pathogen

- Major question: will sources of EFB resistance from Oregon hold up in the eastern USA?
- Why not?
  - Different climatic patterns
  - Higher disease pressure
  - Greater diversity of EFB isolates (?)
    - EFB in Oregon is believed to stem from a single point infection

New Jersey's winter climate is colder than Oregon, but is also somewhat moderated by the Atlantic ocean. Most European hazelnut plants grow well here (minus EFB)

First steps towards answering this question:

- By collecting isolates from around the country and inoculating “so-called” resistant plants under controlled circumstances, it may be possible to answer some questions

- EFB isolates collected from:
  - New Jersey - 2
  - New York - 3
  - Pennsylvania - 3
  - Massachusetts - 1
  - Minnesota - 2
  - Michigan - 1
  - Oregon - 1

12 OR-resistant genotypes challenged with all isolates in separate greenhouse chambers (6 year study):

Final conclusion: only 5 of 12 genotypes showed no signs or symptoms of EFB across all isolates

Corylus avellana

- Ratoli (Spain)
- OSU 495.072 (Russia)

Corylus hybrids

- Grand Traverse (*C. colurna* hybrid - MI, USA)
- OSU 526.041 (*C. heterophylla* hybrid)
- OSU 541.147 (*C. americana* hybrid)

The isolate from Michigan (East Lansing) was able to infect significantly more “resistant” plants than any of the others

Also, it was the only isolate to infect:
‘Gasaway’*, ‘Zimmerman’, and OSU 408.040

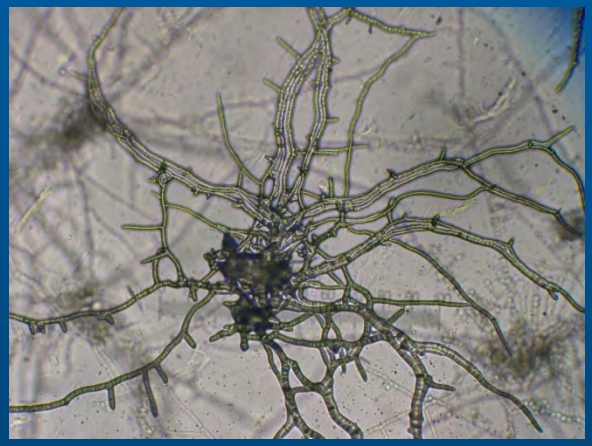
*The ‘Gasaway’ gene for EFB resistance has been widely used in the OSU breeding program—all new orchards are planted to cultivars using this gene for protection. ‘Gasaway’ has been completely resistant to EFB in OR for over 30 years.

- Molnar et al., 2010. Survey of *Corylus* Resistance to *Anisogramma anomala* from Different Geographic Locations. HortScience. 45:832-836

Are their really different races of EFB?

- Currently, we have no definitive means to answer this question. Little is known about the genetics and population biology of *A. anomala*
- As a start, we completed a partial sequencing of the genome of *A. anomala,* largely to develop molecular markers to fingerprint isolates and study its genetic diversity and population structure
  - We have DNA (frozen samples) from over 300 isolates to use in population studies
- However, we learned something interesting along the way

Germinating spores of *A. anomala*



A. anomala culture at 8 weeks

Anisogramma anomala genomics

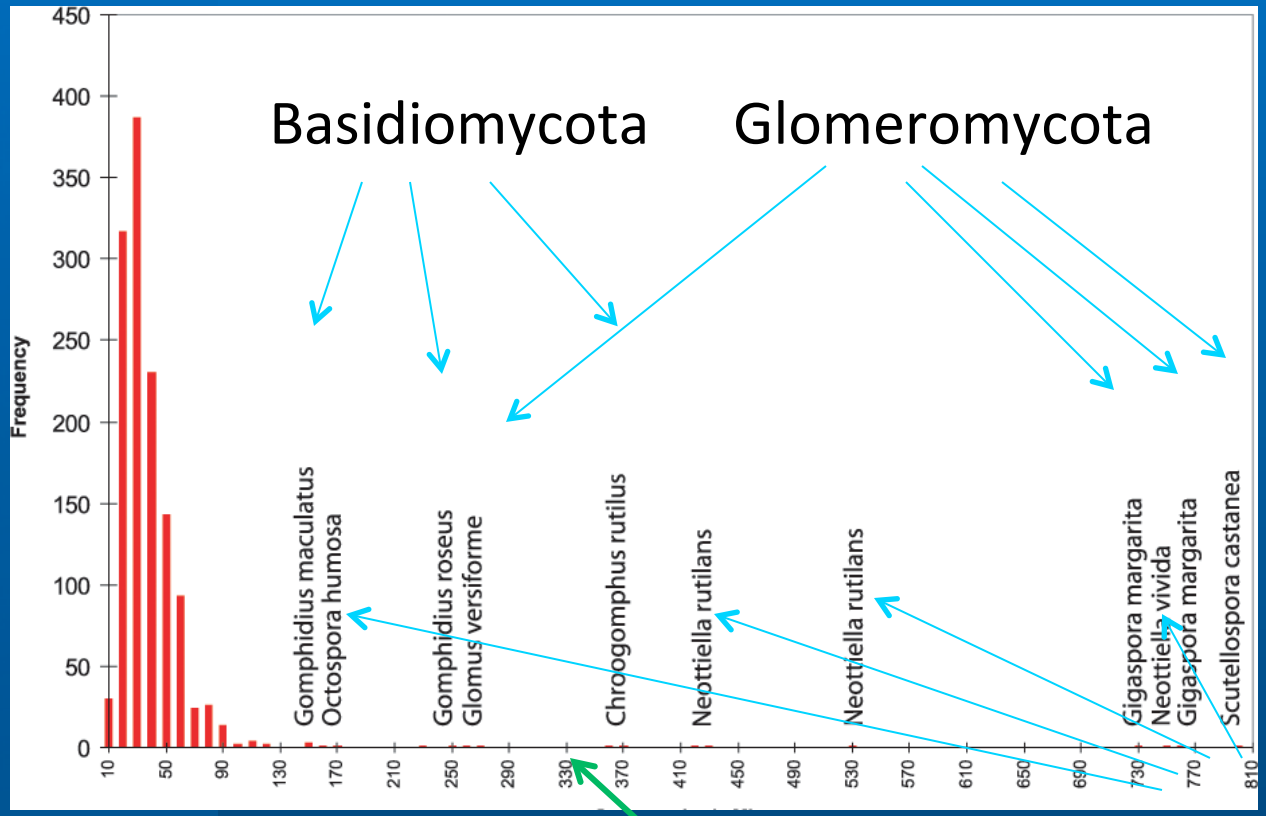
Guohong Cai and Brad Hillman (Rutgers)

A. anomala assembly summary Illumina platform (11x coverage)

	GC ratio	N50	Nmax	Total size
Contigs	**34%**	**4,101**	**31,925**	**340,480,068**
Scaffolds (>= 200bp)	**32%**	**32,987**	**223,344**	**336,895,534**
Contigs after gap-filling	**32%**	**10,384**	**74,811**	**333,579,400**

Huge fungal genome, nearly as large as *C. avellana*! (370 Mb)

Fungal Genome Size Distribution



Genome size was also confirmed by flowcytometry to be >300MB

~85% of the assembled genome is repetitive sequences

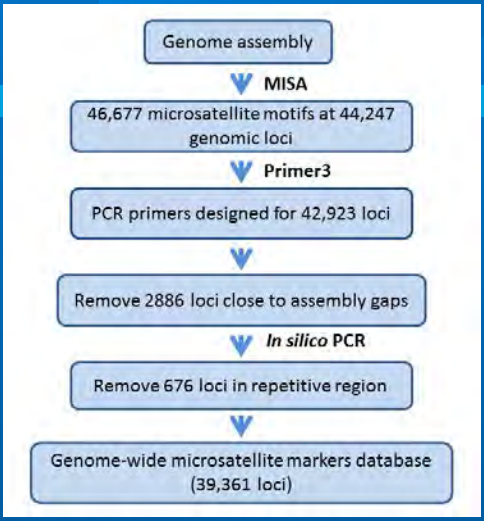
Ascomycota

Anisogramma anomala

Gregory et al, 2007. Nucleic Acids Research 35: D332-D338

Genome-wide microsatellite database

Bioinformatic pipeline

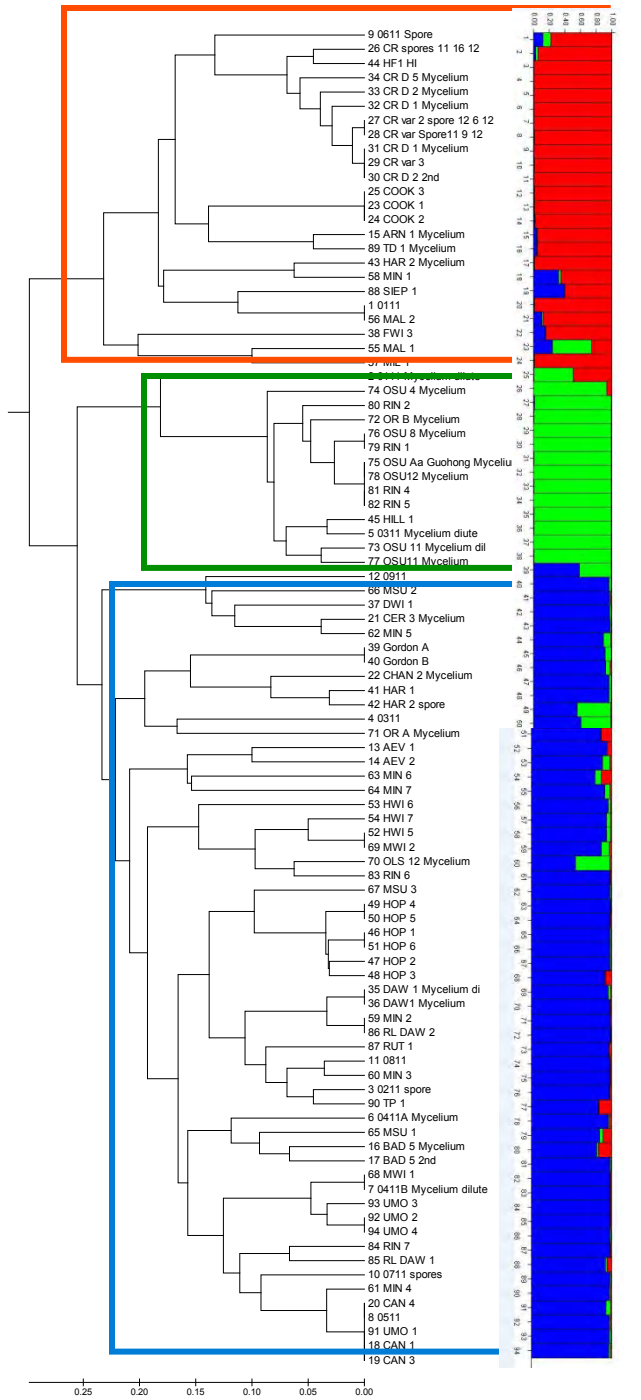


39,361 SSR markers identified

Can mine these to find polymorphic SSR primers

Snapshot of the database

ID	SSR type	SSR	FORWARD PRIMER (5'-3')	Tm(°C)	size	REVERSE PRIMER (5'-3')	Tm(°C)	size	PRODUCT size (bp)
Aa00001	p1	(A)17	TTTTTACTACGAACCGCAAGAT	58.032	22	GAACTTTTATCATAAGCGCTCG	58.248	22	236
Aa00002	p2	(AT)7	TGGATGAAAATAATAAATCGGC	58.033	22	GGTTGGTTTATGAAACAGAATG	56.219	22	234
Aa00003	p1	(A)14	ATGACGTCGTTCTTTTCCTTT	57.853	21	CCCACTATTTTGTACCACTCGT	58.061	22	258
Aa00004	p1	(A)13	AAAGGCGTTTTACAACCAGTTA	57.949	22	TAAACAAGCTTGGAATTCAGGT	57.94	22	200
Aa00005	p1	(T)10	GAACGATCATTTTTGTCCCTTA	58.113	22	TAAATTACTAAGGCGAACCTCG	57.707	22	138
Aa00006	p1	(A)10	TTAACGGGATCGATCATTTATT	57.551	22	TTCTAATATTTAGGAGTGGGCG	57.523	22	264
Aa00007	p3	(TCG)9	ATTATCACAGTTGTCGTCATCG	57.632	22	AACCGGTCTTTAACACCCTATT	58.034	22	238
Aa00008	p1	(A)10	ACTTTTACCAACATTACGGGTG	57.991	22	GGCTTTTCGAATACAAACTGAC	57.939	22	211
Aa00009	p1	(T)10	CGGAATCGTTGTTTATAAGAGC	57.983	22	GTCAGTAAAGCCTAACAGTGCC	58.086	22	230
Aa00010	p2	(AT)6	CAAAACTTGATGGTAAGGAGGA	58.238	22	CAACAAAGGCTTCGCTTATAGT	57.815	22	224
Aa00011	p2	(TA)6	TTTTTATTGACTGATGTTGCAAGT	57.949	24	GAACTTGAATTGAAAACCCGTA	58.163	22	236
Aa00012	p1	(T)21	CCATATATGTAATGTTTCCCCC	57.169	22	TGGCCAATTAATGTTTGTGATA	57.973	22	276
Aa00013	p1	(A)11	GTTAGGGTCTTTACCACCATCA	57.983	22	GTCCTTACGCCAATATTACCAA	58.091	22	248
Aa00014	c	(A) 10tggaatattaa (T)11	ACGCTTCTTGGATAGAGAACTG	57.778	22	GAGAACCCTCACTTGATCAGAA	58.377	22	201
Aa00015	c	(T) 10ccatttaccga caaagaccttggg actacgtattctttc gcta(T)10	TGGTTATTGTTGAGAACGTTGA	58.201	22	TGGAAAGCTCCATTTCAATAGT	57.888	22	204
Aa00016	p1	(A)11	ACTCCTCCTTTCGCTAAATCTT	57.768	22	TCCAATTAAGGAGGGGTATTTT	57.978	22	156
Aa00017	p1	(A)11	ACTTAACACACTGTCAAACCCC	58.018	22	TTTAGGGCATAAGATACGCATT	57.893	22	235
Aa00018	p1	(T)10	GCACGTAACGGGTTAGGTATTA	58.164	22	GGTGAATCCTTTTCGATAGTGT	57.233	22	259
Aa00019	p1	(A)10	TCTTAAGAACATGAAGTTTTGGAA	57.228	24	TCCGGGTTATACTGGAAATAAT	56.666	22	245
Aa00020	p1	(T)12	TCCTCTCGGTAAAGGTTATTGA	57.889	22	CAAACAGGCGAAAAGGATATAA	58.331	22	278
Aa00021	p1	(T)11	CACATTAACGCTGTGTGTCTTT	57.896	22	TTATGCTAGACATTAGGGCGAT	57.957	22	264
Aa00022	p1	(A)10	ACGACCTATTTTTCTCAACGAC	57.433	22	AAAGTTCCTGTATGTTAGGCCA	57.828	22	224
Aa00023	p3	(GTA)13	TAAGGTATAGGGTGAAGCCAAA	57.85	22	CACAGGATTTTTAGGGTGTCAT	57.983	22	265
Aa00024	p1	(T)15	TATAGGGATGATTATCGAACGG	57.992	22	CTTCGAGGGAATGTAAATATGG	57.662	22	244
Aa00025	p1	(T)13	ACGAATTCTACGTAACGACCTG	58.338	22	AAACGTCGAATTGTTGTTTTCT	57.853	22	248
Aa00026	p1	(A)10	ACACGGTGTTATCGAAAGAGTT	57.804	22	TTTTTACGGGTTGTTTCTTGTT	57.792	22	272
Aa00027	p1	(T)12	CACCAAATATTGAACGCTTTTT	58.248	22	TTTCCTAAGAAGCCAAATTACG	57.682	22	270
Aa00028	p1	(T)10	GGTCAGAAACAACTCGAATCAT	58.174	22	GAAACGATTTCAAATGAACGAT	58.113	22	207
Aa00029	p1	(A)11	TTTGGTGTAAAGGTTAGGTTCG	58.237	22	ACGAAGGTATCGCTATTGAAAA	57.983	22	234
Aa00030	p2	(TA)6	GTCAGCTCTTGAGGATTGAAAC	58.055	22	ACAAGTCCTCTTGCGAAAATAA	58.077	22	214
Aa00031	p1	(A)14	GTAGAAATAACCCGTCAACGAA	58.188	22	AAAACATGTCGTCGTTGTAATG	57.628	22	272
Aa00032	p1	(A)10	GGAATTGAAAGGGATCTAGCTT	57.935	22	CACTTATATTGCAGTCCCACAA	57.719	22	226
Aa00033	p1	(A)11	GCGTTGACGTTTAGAAGCTAAT	57.814	22	CCACAAAGGAACTTTCAGACTC	57.964	22	277
Aa00034	p1	(A)11	ATCACCTTTTGAATATGGATCG	57.948	22	TGGTTCCTTTTACACTTGAAGG	58.288	22	263
Aa00035	p2	(TA)9	GGGTGACATACAGTTAACCCAC	58.227	22	GTAGACTCATAATCCCCTTCCC	57.977	22	150
Aa00036	p1	(T)12	TAAACTTCAAATTAAACCGCGT	57.967	22	CGAAATTGTAACCAAAAGAAGC	58.008	22	212
Aa00037	p1	(T)10	AGTCTAGGGTCATATTGGCGTA	57.8	22	TCACTGCAAAAACAACAAAGTC	57.989	22	266

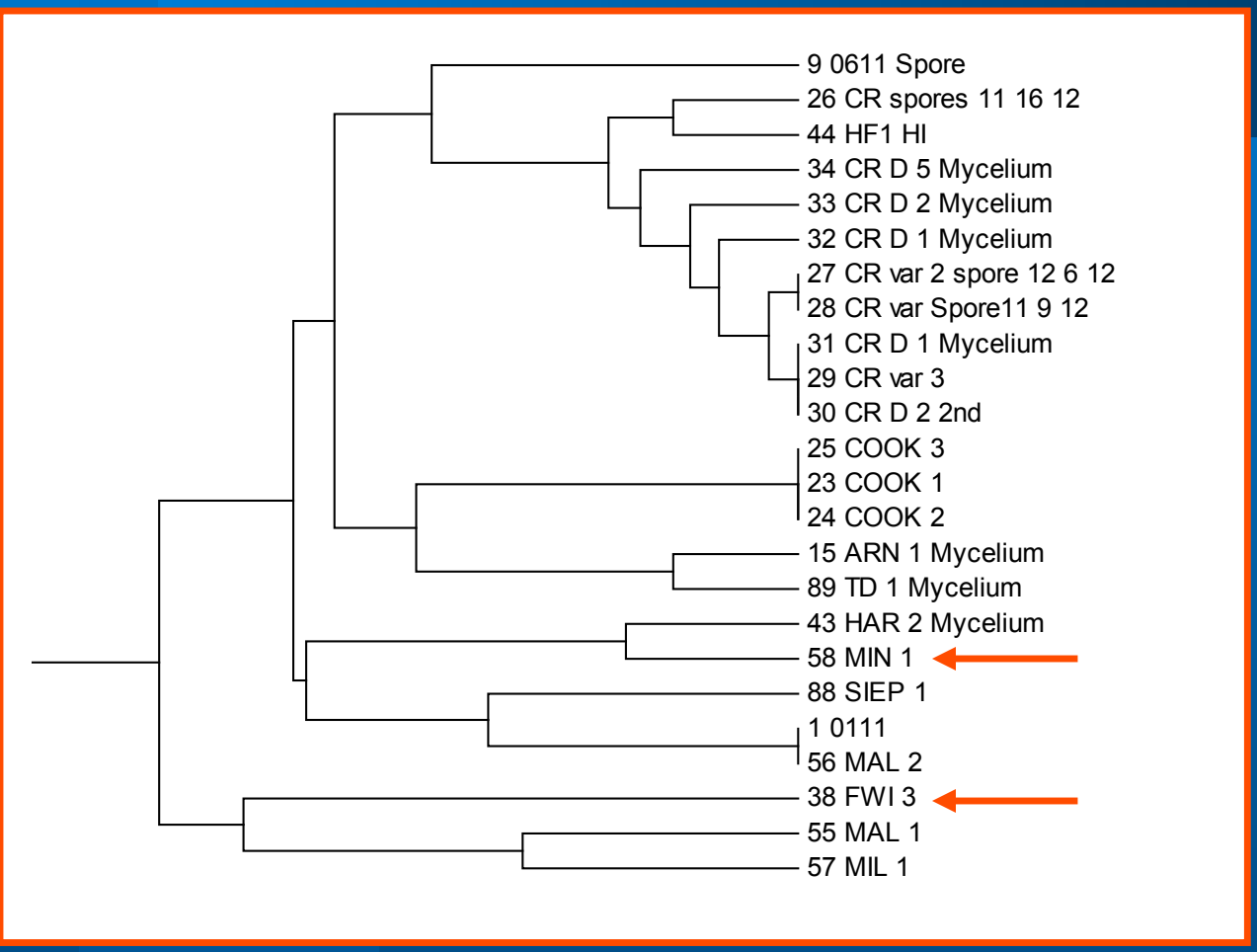


First glimpse of *A. anomala* population structure:

Combination of two statistical analysis show support (UPGMA and STRUCTURE)

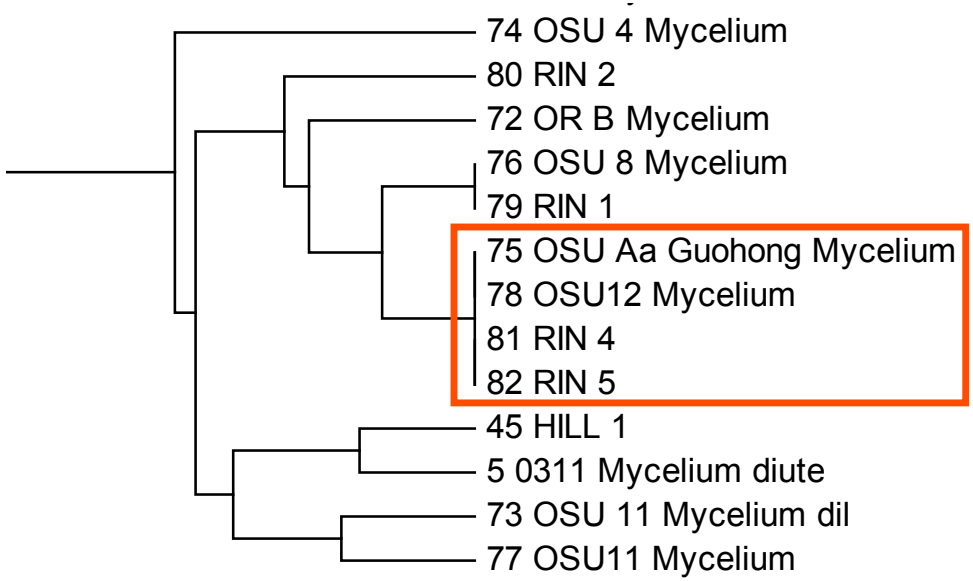
- 93 isolates (about 50-60 more will be included)
  - From NJ, PA, NY, OH, MA, ME, MN, WI, MI, IL, MO, Ontario, P.E. Island, etc…
- 16 SSR markers (~5 more for final study)
- Red clade is mostly NJ and eastern PA isolates
- Green clade is Oregon and one MI isolate (Leslie, MI)
- Blue clade represents many isolates from PA across through MI

New Jersey - eastern clade

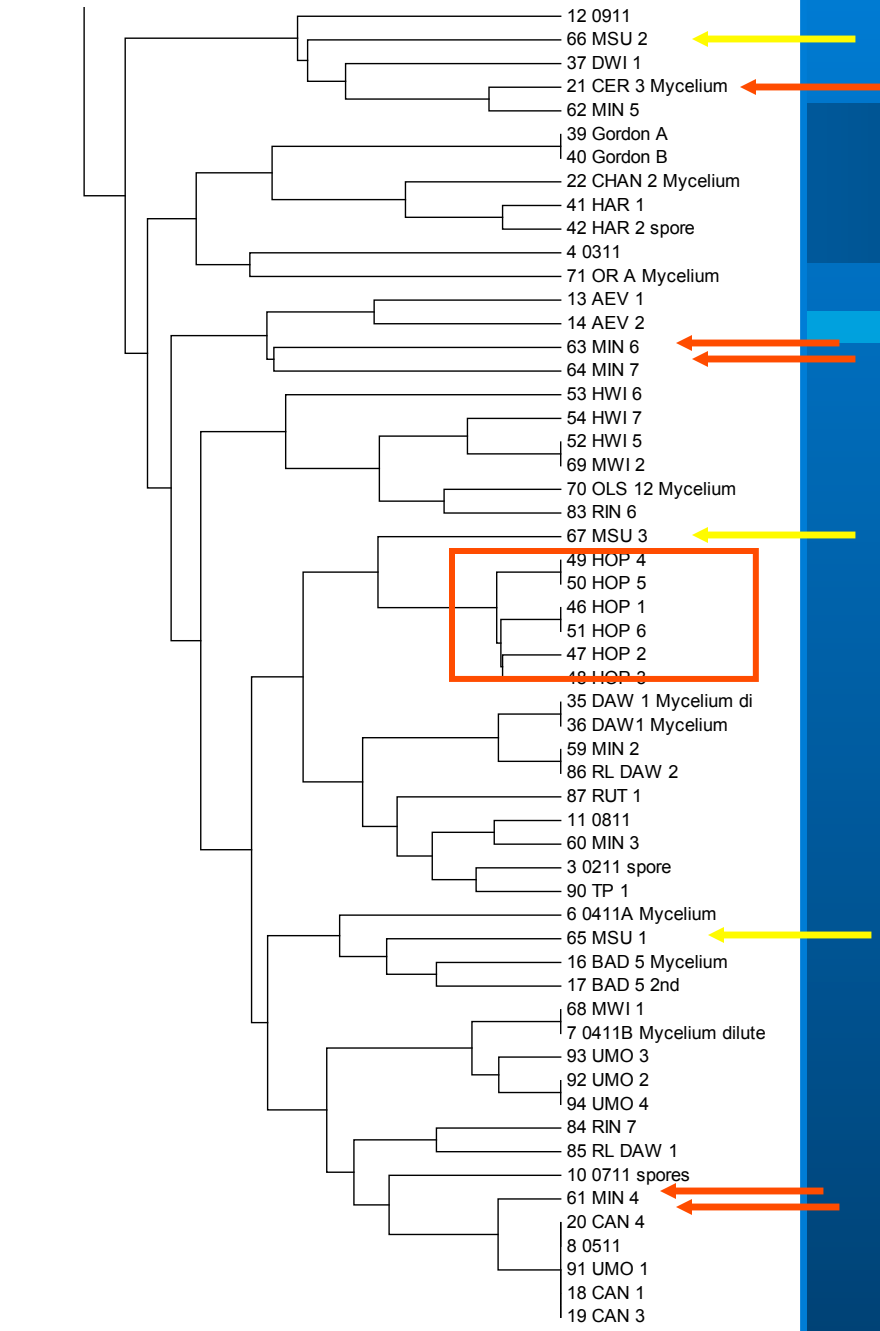


- Distinctly separate from all other isolates.
  - Isolates in this clade found nowhere else in dendogram
- One MN isolate, one WI isolate found in this clade
- Adding more isolates to study might shed light on what is going on here

Oregon clade



- All 8 OR isolates fell in this distinct clade
- Interestingly, most isolates from Leslie, MI (RIN) fell in this clade too
  - several appear identical
- Is this the origin of the OR introduction
  - More likely, this farmer bought infected plants from Oregon!
  - Need more isolates to tell a better story

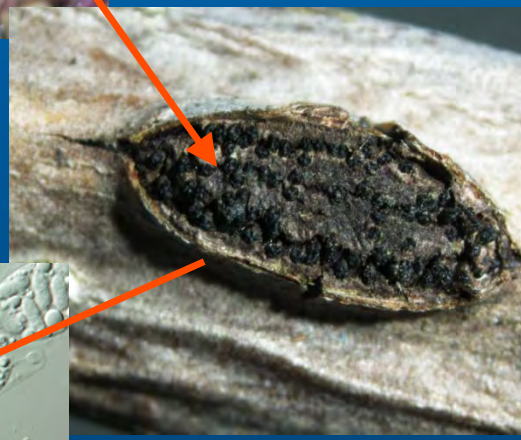


Midwestern clade

- A wide spread of isolates from diverse geographic origins fell into this clade (more similar to each other than either NJ or OR clade)
- Multiple isolates per location:
  - ME, MI (4), WI (5), MN (3), NY (2), PA (4), OH (1), MO (1), Toronto (1), PE island (1).
- Some locations were tightly clustered
  - Hopbottom, PA
- Others were widespread in this clade
  - Roy Cerling, Wycoff, MN
  - East Lansing, MI
- Adding more isolates will help us to better understand *A. anomala's* population structure

SSR study confirmed *A. anomala* is homothallic

- The EFB fungus only produces sexual ascospores (no asexual conida like many other fungi)
- We extracted DNA from spores from single stromata
- SSR profiles showed they were haploid and uniform (no diversity within a stomata)
  - Resulting offspring identical to parent isolate
- Confirms *A. anomala* is a homothallic (self-pollinating) fungus
- Should see a much reduced level of recombination, which when combined with its long life-cycle suggests a slow rate of change
  - In SSR profile, we see a limited amount of diversity compared to many other organisms



http://umaine.edu/ipm/files/2010/11/200402978HiattCorylusb6.jpg

Rutgers Breeding program:

- Started in 1996, we have around 35,000 trees in the field from controlled crosses and new germplasm introductions
- The breeding programs continues: 10 years ago we had very few EFB resistant selections with decent kernel quality
  - Today we have many thousands of seedlings that meet these criteria. The challenge is now identifying the best plants for clonal propagation
- From our earliest efforts, we selected 14 plants showing excellent qualities:
  - Highly EFB resistant; medium to large size nuts, with over 50% kernel by weight; round kernels; high crop loads, few kernel defects

14 EFB-Resistant selections being propagated for testing:

ID Number	Resistance source	Kernel Characteristics (mm) 10 kernel ave. Height	Length	Width	*ave. kernel weight (g)	*ave. kernel %
CRXR09P32	Grand Traverse	15	13	14	1.3	53.5
CRXR10P69	Grand Traverse	15	12	14	1.4	57.7
CRXR11P07	Grand Traverse	16	13	14	1.3	50.2
CRXR11P10	Grand Traverse	15	12	13	1.3	52.3
CRXR12P35	Grand Traverse	14	13	14	1.2	51.5
CRXR04P43	Ratoli	14	12	13	1.0	57.8
CRXR06P56	Ratoli	16	11	13	1.0	47.7
CRXR03P26	Yoder #5 ('Rush')	17	12	13	1.2	45.0
CRXR03P70	Yoder #5 ('Rush')	17	12	13	1.4	52.4
CRXR07P58	Yoder #5 ('Rush')	14	14	15	1.4	45.5
CRXR08P24	Yoder #5 ('Rush')	15	13	14	1.3	44.8
CRXR11P47	Yoder #5 ('Rush')	15	12	12	1.0	55.3
CRXR11P48	Yoder #5 ('Rush')	14	12	13	1.1	57.3
CRXR11P43	Zimmerman (Gas.)	20	13	14	1.1	53.6

Barcelona: kernel is 1.6 g, kernel % is 44.2
Lewis: kernel is 1.1 g, kernel % 47.4

* 20 nut average yearly, over 2 or 3 years data


Clonal Yield Trial at Rutgers
July 2011 (established 2009)
• Trials must be evaluated for at least 7 years
• Testing in multiple locations with different climates and soils will help us identify the best plants and to determine if any are suitable for larger scale propagation and release

Trials located at:

- Rutgers (2009 and 2010; New Brunswick, NJ)
- University of Nebraska, Lincoln (2009)
- University of Guelph, Ontario, Canada (2011)
- Malcolm Olsen (2010; Findley Lake, NY—western NY)
- Jeff Zarnowski (2010; Cortland, NY—central NY)
- Peter Haarmann (2010; Aquebogue, NY—eastern Long Island)
- Tucker Hill (2009/2010; Etters, PA)
- Shuster Farms (2011; Stockton, NJ—western NJ)
- Ruscke Farms (2011; Millville, NJ—southern, NJ)

A significant expansion of hazelnut production is on the horizon!

- Eastern filbert blight is not the limiting factor it once was
- While a number of questions still need to be answered, collectively we have all the pieces of the puzzle required to breed for well-adapted, productive plants
  - EFB-resistant *C. avellana* might work well in the Mid-Atlantic/Fruit belt region
  - Interspecific hybrids are needed for production in the more stressful upper Mid-West
  - plants can be developed for different market applications
- Enhanced collaboration between hazelnut researchers in North America can expedite the development of hazelnuts as a more widely grown crop


Questions?

Acknowledgments and thanks!

- New Jersey Agricultural Experiment Station
- Rutgers Center for Turfgrass Science
- USDA ARS Clonal Germplasm Repository
- USDA-NIFA Specialty Crops Research Initiative Competitive Grant 2009-51181-06028
- Northern Nut Growers Association
- Contributors: John Capik, Reed Funk, Megan Muelhbauer, Josh Honig, Shawn Mehlenbacher, Guohong Cai, Ning Zhang, Adam Morgan, David Zaurov, Brad Hillman, Katie Morey, Loren Isom, Milford Hanna, Adam Howard, Scott Josiah, Roy Cerling, Lois Braun, Phil Rutter, Dennis Gibson, Emily Walsh

RUTGERS
New Jersey Agricultural Experiment Station

Center for Turfgrass Science
RUTGERS

Hybrid Hazelnut Consortium partners:

OSU
Oregon State University

University of Nebraska Lincoln

Arbor Day Foundation