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Survey of Hazelnut Cultivars for Response to Eastern Filbert Blight Inoculation

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Abstract. **Ninety hazelnut (*Corylus* sp.) genotypes were surveyed for response to the eastern filbert blight pathogen [*Anisogramma anomala* (Peck) E. Müller] following greenhouse inoculation using a combination of enzyme-linked immunosorbent assay (ELISA) and visual inspection for cankers. Most were cultivars of the European hazelnut (*Corylus avellana* L.) and a few were interspecific hybrids. Six genotypes did not display signs of the pathogen or symptoms of disease: 'Closca Molla', 'Ratoli', 'Yoder #5', 'Potomac', 'Medium Long', and 'Grand Traverse'. 'Closca Molla' and 'Ratoli', both minor Spanish cultivars, are superior in many respects to 'Gasaway', which has been extensively used as a completely resistant parent in breeding. 'Potomac' and 'Yoder #5' have *C. americana* Marsh. in their pedigrees, 'Grand Traverse' is one-quarter *C. colurna,* and the origin of 'Medium Long' is uncertain. The random amplified polymorphic DNA (RAPD) marker generated by primer UBC 152, which is linked to the single dominant resistance gene of 'Gasaway', is absent in these six genotypes, and thus they appear to be novel sources of genetic resistance to this devastating disease.**

Oregon's Willamette Valley is the top producer of European hazelnuts in the United States, accounting for 99% of the U.S. crop and 3% to 5% of the world crop (Mehlenbacher and Olsen, 1997). Eastern filbert blight (EFB), caused by the pyrenomycete *Anisogramma anomala* (Peck) E. Müller, is a serious and widespread disease in the Willamette Valley. This disease can destroy the productivity of a moderately susceptible cultivar in 8 to 12 years (Johnson et al., 1996), so its control is an important industry goal. Because of the expense of fungicides and detrimental effects on yield caused by severe pruning of cankers, genetic resistance is an especially appealing approach to fighting this disease (Mehlenbacher, 1995).

Developing cultivars for the kernel market that are completely resistant to EFB is an important goal of the Oregon State Univ. (OSU) breeding program (Mehlenbacher, 1995). For this market, hazelnuts must meet strict standards for size, shape, shell thickness, ease of pellicle removal, and flavor (Mehlenbacher, 1994). Most of the resistant material in the OSU hazelnut breeding program carries the single, dominant, resistance gene found in the obsolete pollinizer 'Gasaway' (Mehlenbacher et al., 1991). 'Gasaway' has deficiencies in nearly all of these characteristics. Resistant genotypes with good nut quality would be beneficial to the breeding program.

Marker-assisted selection is one tool that breeders can use for early selection; random amplified polymorphic DNA (RAPD) markers are one of the least expensive, rely on the polymerase chain reaction (PCR), and are well suited to the high throughput of breeding programs (Welsh and McClelland, 1990; Williams et al., 1990). Short primers (usually 10 bp) bind to short, inverted repeats that amplify random sequences. These amplified DNA fragments are subjected to electrophoresis and later visualized by ethidium bromide staining. Davis and Mehlenbacher (1997) used bulked segregant analysis (Michelmore et al., 1991) to identify five RAPD markers linked to the 'Gasaway' resistance gene. One of these markers (UBC 152_{800}) is robust to amplification conditions, easy to score, and routinely used to select resistant seedlings in segregating populations (Mehlenbacher and Lunde, unpublished). (Note that primer names are followed by subscripts denoting the size of the polymorphic band that is amplified.)

Concern exists about the durability of the 'Gasaway' source of resistance (Pinkerton et al., 1998). Cultivars differ in level of susceptibility (Pinkerton et al., 1993), but the complete resistance desired by the industry would eliminate the cost of chemical control. Thus, new sources of genes conferring complete resistance would be highly beneficial. The purpose of this study was to survey germplasm from the collections of the OSU breeding program and the U.S. Dept. of Agriculture, Agricultural Research Service (USDA, ARS) National Clonal Germplasm Repository (NCGR), Corvallis, Ore., for response to inoculation by the EFB pathogen.

Materials and Methods

Plant materials. Three scions of each of 90 hazelnut selections from the NCGR and the collection of the OSU hazelnut breeding program (Table 1) were gathered in Dec. 1996, stored at 0 °C, and grafted onto *C. avellana* rootstocks in Spring 1997. 'Gasaway' was included as a resistant control and 'Ennis' and 'Daviana' as susceptible controls. Grafted trees were placed in 3.7- or 5-L pots in a mix containing equal volumes of peat, pumice, and fine bark dust. In addition, 9 g of Sierra 3–4 month release fertilizer (17N–2.6P–10K with micronutrients) was added to each pot. Supplemental fertilizer (Peters' 20N–8.7P–16.6K) was applied as needed. Plants were grown in a glasshouse under 24 °C day/18 °C night until they were ready for inoculation. For genotypes scored as resistant in the first year's inoculations, scions were recollected, grafted, inoculated, and retested in 1998. Genotypes whose grafts failed the first year were also regrafted, inoculated, and assayed in the second year.

Inoculations. Inoculations were started ≈3 to 5 weeks after grafting, once shoots had four to five nodes (Coyne et al., 1996). All actively growing branches (usually two or three) of the grafted trees were marked with tape three or four nodes from the apical meristem to indicate the point of inoculation. Shoots were inoculated with a suspension of 1×10^6 spores/mL in distilled water (Johnson et al., 1994). Chamber frames were constructed of polyvinyl chloride tubing (1.27-cm diameter), placed on top of a bench (1.22 × 3.44 m) and covered with white 4-mil (0.1-mm) polyethylene sheeting. About 75 trees were placed in each of two inoculation chambers. A humidifier was placed in each chamber, the chamber was closed, and the plastic was fastened with clothespins. The humidifiers were programmed to run from noon to 6 PM and from midnight to 4 AM. The chambers were opened 5 d after inoculation, left open for 2 d, and then inoculations were repeated. Each tree received a total of three inoculations. They remained in the glasshouse with optimal watering for 6 months prior to assaying for infection.

Infection assays. A combination of three assays was used to detect the presence of *A. anomala* in inoculated shoots. An enzyme-linked immunosorbent assay (ELISA) was performed on inoculated shoots of two grafted trees of each genotype as described by Coyne

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et al. (1996), except that Nunc Maxisorp 96-well MicroWell Microplates (Fisher Scientific, Pittsburgh) were used instead of Corning microtiter plates. The third grafted tree of each genotype was inoculated as above and transported to the Southwest Washington Experiment Station, Vancouver, Wash.; planted in a nursery row in Feb. 1997; and evaluated for canker incidence in June 1998. Trees that became infected in either of the two assays were scored as susceptible. Genotypes scored as resistant in the first year were retested the second year, using the same inoculation procedures and infection assays, except that tissue was sectioned and examined microscopically for the presence of hyphae in samples that gave inconclusive results with ELISA. Hyphae were stained with 0.05% trypan blue in lactophenol and observed with a light microscope (Stone et al., 1992).

DNA extraction and RAPD screening. In the spring, DNA was extracted from the very young apical meristem and the first leaf of field-planted trees. When samples were needed later in the season, DNA was extracted from catkins, as Cheng et al. (1997) had successfully extracted DNA from this tissue. The sampled tissues were ground in the laboratory within an hour of field collection. The DNA extraction protocol was based on that of Davis et al. (1998). A leaf juice press (MEKU, Wennigsen, Germany) was used to grind leaf samples with an extraction buffer consisting of 0.35 M sucrose, 100 mM Tris, 50 mM potassium chloride, 25 mM EDTA, and 5% PVP (mol. wt. 40,000) dripping onto the grinding bits. About 500 µL of the macerate was collected in a 1.7-mL centrifuge tube on ice, and the grinding bits were thoroughly rinsed between samples. Each sample was then centrifuged for 5 min at 17,900 g_n. The supernatant was discarded and the pellet was resuspended in 640 µL lysing buffer [78.3 mM EDTA pH: 8.0, 39.16 mM Tris-HCl at pH 8.0, 2.12% n-lauroylsarcosine, 2.65% Triton X-100®, and 10.7 $\mu g \cdot \mu L^{-1}$ Proteinase-K (Fisher Scientific), which was added just before use]. Samples were incubated for 1 h at 37 °C in a shaker at ≈160 rpm. After centrifugation at 17,900 g_n for 5 min, 500 µL of supernatant was transferred to a new tube. An equal volume of cold isopropanol was added, mixed, then tubes were stored in the freezer at –20 °C for at least 30 min. Tubes were centrifuged at 17,900 g_n for 5 min, the supernatant was discarded, and the remaining liquid was aspirated. The pellet was resuspended in 220 µL of 1× TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) overnight at 4 °C. Samples were extracted with 25 phenol : 24 chloroform : 1 isoamyl alcohol by vortexing 10 s and then centrifuging for 10 min. A 150-µL aliquot of the aqueous phase was transferred to a new tube and precipitated with two volumes of 95% EtOH : 3 M sodium acetate, pH 5.5 (20:1) at –20 °C for at least 30 min. The tubes were centrifuged, the supernatant was poured off, and the pellet was washed with 70% EtOH. The tubes were centrifuged for 5 min, and the supernatant was discarded. The pellet was air-dried overnight and resuspended in 500 µL 1× TE buffer.

PCR assays. The PCR reactions were performed in a volume of 15 µL containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.15% Triton X-100, 1.5 mM $MgCl_2$, 120 µM each of dATP, dCTP, dGTP, and dTTP, 0.1 µM of primer, 3–25 ng of DNA template, and 0.75 units of Taq polymerase (Promega, Madison, Wis.) (Davis and Mehlenbacher, 1997). Ninety-six reactions were run simultaneously using a Geneamp® PCR System 9700 thermal cycler (Perkin-Elmer Corp., Foster City, Calif.). Genotypes that had been scored as resistant, including 'Gasaway', as well as a control containing no DNA, were tested. The thermal cycler program consisted of denaturation for 1 min at 94 °C, followed by 5 cycles of: 1 min at 94 °C, 90 s at 37 °C, 30 s at 54 °C and 2 min at 72 °C. This was followed by 35 cycles of 15 s at 94 °C, 45 s at 37 °C, 30 s at 54 °C, and 90 s at 72 °C. Primer extension was for 7 min at 72 °C and then samples were held at 4 °C until they were retrieved. The DNA from each tree was amplified using primer UBC 152 (Univ. of British Columbia, Vancouver). Amplification products were separated by electrophoresis on 1.5% agarose gels, and stained with ethidium bromide. They were visualized with a transilluminator and photographed.

Results and Discussion

Of the 90 cultivars (Table 1), six, varying widely in agronomic quality, resisted infection by *Anisogramma anomala*. Desired traits

Table 1. Response of hazelnut cultivars to inoculation with *Anisogramma anomala*.

Cultivar	Origin	Accession (CCOR)[z]	Cultivar	Origin	Accession (CCOR)[z]
		Noninfected			
Closca Molla	Spain	257	Potomac	USA–Maryland	377
Gasaway[y]	USA–Wash.	54	Ratoli	Spain	344
Grand Traverse	USA–Mich.	559	Yoder #5	USA–Ohio	---
Medium Long	USA–N.Y.	701			
		Infected			
A Pellicola Bianca	Italy	454	Louisen's Zeller	Germany	207
Alcover	Italy	375	Ludolph's Zeller	Germany	330
Amandi	Spain	566	Martorella	Spain	444
Apolda	Italy	360	Molar	Portugal	488
Ata Baba	Azerbaijan	480	Mogulnuss	England	324
Atlas	Denmark	389	Morell	Spain	6
Aveline d'Angleterre	England	387	Multiflora	England	322
Bandnuss	England	382	Napoletana	Italy	374
Barcelloner Zeller[x]	England	331	Napoletanedda	Italy	463
Bard	England	514	Noce Lungha	Italy	296
Barr's Zeller	England	333	Nociara	Italy	385
Bearn (Du Bearn)	France	461	Nottingham	England	297
Blumberger Zeller[x]	Germany	205	Palaz	Turkey	29
Brixley's New	USA–Oregon	288	Pallagrossa	Italy	372
Burchardt's Zeller	Germany	334	Pearson's Prolific	England	335
Buttner's Zeller	Germany	329	Pere Mas	Spain	340
Ceret	Spain	508	Pinyolenc	Spain	339
Cherkesskii II	Russia	544	Pioneer	Russia	548
Comen	Italy	362	Planeta	Spain	445
Comun	Portugal	486	Princess Royal	England	327
Culpla[x]	Spain	255	Prolific Closehead	England	326
da Viega	Portugal	487	Purple Aveline	England	---
Daviana[w]	England	42	Quiros	Spain	279
Des Anglais	England	481	Ratllada	Spain	442
Ennis[w]	USA–Wash.	11	Red Filbert	England	317
Espinaredo	Spain	509	Reed	USA–Maryland	383
Faroka[x]	Canada–B.C.	---	Restiello	Spain	280
Frizzled Filbert	England	218	Riekchen's Zeller	Denmark	393
Fructo Albo	Italy	511	Ros de la Selva	Spain	260
Garibaldi	England	338	Rossetta	Spain	379
Garrofi	Spain	341	Sant Joan[x]	Spain	271
Gironell (Grossal)	Spain	---	Sant Pere	Spain	270
Grifoll	Spain	443	Sickler's Zeller	Germany	321
Gunslebener Zeller	Germany	382	Sodlinger	Italy	459
Gustav's Zeller	Germany	206	The Shah	England	319
Heynick's Zeller	Germany	390	Tomasina	Spain	441
Istarski Duguljasti	Slovenia	272	Tonda Bianca	Italy	21
Jean's	Italy	264	Tonda Rossa	Italy	267
Jeeve's Samling	England	352	Truchsess Zeller	Germany	328
Kadetten Zeller	Germany	323	Ugbrooke	New Zealand	245
Kunzemuller's Zeller	Germany	353	Volle Zeller	Germany	---
Lange Landsberger	Germany	325	Webb's Prize Cob	England	336
Liegel's Zeller	Germany	316	Witpit Lambertsnoot	Netherlands	573

[z]Corvallis *Corylus* (CCOR) accession number assigned by USDA, ARS National Clonal Germplasm Repository, Corvallis, Ore.
[y]Resistant control.
[x]All grafts failed in 1996; regrafted and tested in 1997. Disease response observed in 1997.
[w]Susceptible controls.

for the kernel market are: medium size, round shape, 50% kernel or better, about two nuts per cluster, very little fiber on the kernel pellicle, good blanching ability, and few defects (<35%). Nuts must fall free from the husk because they are mechanically harvested off manicured orchard floors in Oregon. These six cultivars should be useful as parents in the development of new, resistant varieties.

Corylus avellana cultivars. 'Closca Molla' and 'Ratoli', both from Spain, have acceptably round kernels that blanch fairly well. 'Closca Molla' has thin shells (and hence high percentage of kernel) but, unfortunately, low yield, and 'Ratoli' has been recommended as a potential variety in Spain because of its desirable kernel traits (Tasias-Valls, 1975). These cultivars may have potential for immediate use as cultivars in Oregon, but have not yet been included in replicated yield trials.

Interspecific hybrids. At least three of the other four clones are related to species other than *C. avellana*. 'Grand Traverse' is listed as being the offspring of a cross between 'Faroka' (*C. colurna* L. x *C. avellana*) and 'Royal' (*C. avellana*) made by Cecil Farris (Farris, 1989). 'Faroka', selected by J.U. Gellatly in the 1950s from seedlings grown from seed collected from a Turkish tree hazel (*C. colurna*) growing near European hazels, phenotypically appears to be an interspecific hybrid. Thus, 'Grand Traverse' would be one-quarter *C. colurna*, which partly explains its lack of precocity. Resistance to natural inoculation in this variety was previously reported by Farris (1989). Interestingly, the parent 'Faroka' was infected in our tests, and 'Royal' has been highly susceptible in commercial orchards (Cameron, 1976). The incompatibility alleles of 'Grand Traverse' (S_{11} S_{25}) indicate that it inherited S_{11} from 'Faroka', but neither allele of 'Royal' (S_1 S_3) is present. This indicates that 'Royal' is not the pollen parent of 'Grand Traverse', and that its EFB resistance may have come from its unknown pollen parent. 'Potomac' is a *C. americana* x *C. avellana* hybrid, and the appearance of 'Yoder #5' clearly indicates that it has *C. americana* in its pedigree. Neither of these varieties blanches well. 'Yoder #5' has the added deficiencies of being low-yielding and quite sensitive to big bud mite (primarily *Phytoptus avellanae* Nal.). Resistance to EFB has been previously reported in American–European hybrids (Coyne et al., 1998) but its genetic control has not been clarified. 'Potomac' is the result of a cross between 'Rush' (a *C. americana* selection) and 'DuChilly', a European hazelnut cultivar. There are conflicting reports about whether 'Rush' itself is susceptible to EFB (Slate, 1947; Thompson et al., 1996). 'Reed' ['Rush' x 'Bolwyller' (syn. 'Hall's Giant')] has the same *C. americana* parent, but was susceptible in the greenhouse inoculation. Other selections from 'Rush' x *C. avellana* were resistant in previous tests (Coyne et al., 1998). The parentage of 'Medium Long' is questionable. Some of its ancestors may have included *C. avellana* cultivars imported from Europe, but its resistance to EFB suggests that it may be an interspecific hybrid. We obtained this selection from the New York Agricultural Experiment Station, Geneva, where the European hazel was routinely crossed with *C. americana* and most selections were interspecific hybrids (Slate, 1947).

Release of completely resistant, late-shedding pollenizers is also an objective of the breeding program. These six resistant varieties have diverse self-incompatibility alleles: 'Closca Molla' (S_2 S_5), 'Ratoli' (S_2 S_{10}), 'Grand Traverse' (S_{11} S_{25}), 'Medium Long' (S_{11} S_{12}), 'Potomac' (S_5 S_{12}), and 'Yoder # 5' (S_{10} S_{23}). Incompatibility should not be a barrier to using these cultivars in breeding, and their potential for use as pollenizers deserves attention.

The expected release of varieties carrying the 'Gasaway' gene for resistance to EFB and the nature of the causal fungus indicate that multiple sources of resistance to this disease will be needed for durability. These six cultivars do not amplify the UBC 152_{800} marker that is linked to EFB resistance in 'Gasaway' and its progeny, and hence appear to be new sources of genetic resistance. If this is so, the release of new resistant cultivars developed from these six genotypes should provide insurance against new virulence in the pathogen. By deploying varied sources of genetic resistance to EFB, selection pressure for races of *Anisogramma anomala* able to overcome the 'Gasaway' resistance gene will be lower.

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