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Survey of Hazelnut Germplasm from Russia and Crimea for Response to Eastern Filbert Blight

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Abstract. **Six hundred five hazelnut (*Corylus avellana* L.) seedlings from a diverse germplasm collection made in the Russian Federation and the Crimean peninsula of the Ukraine were inoculated with the eastern filbert blight (EFB) pathogen *Anisogramma anomala* (Peck) E. Müller and their responses evaluated. Responses were rated on a scale of 0 to 5, in which 0 represents no sign of EFB and 5 represents all branches exhibiting cankers. At final evaluation, eight seedlings showed no signs of the pathogen or symptoms of the disease. Five additional seedlings expressed only very minor signs of the pathogen (rating = 1). The remainder ranged in disease expression from moderately to severely infected to dead with 89.7% (470 of 524) of the surviving seedlings rating 4 or 5. Of the 13 apparently resistant seedlings (rating 0 or 1), seven originated from nuts purchased from roadside vendors near Simferopol, Crimea, Ukraine; five from nuts purchased at an outdoor market near Krasnodar, Russia; and one from nuts obtained from the hazelnut breeding program of the Nikita Botanical Gardens, Yalta, Crimea, Ukraine. Random amplified polymorphic DNA (RAPD) markers generated by the primers UBC 152_{800} and OP $AA12_{850}$, which are tightly linked to the single dominant resistance gene 'Gasaway', were not present in all 13 resistant seedlings, providing support, along with their geographic origins, that they represent novel sources of genetic resistance to EFB.**

Eastern filbert blight (EFB), incited by the fungus *Anisogramma anomala* (Peck) E. Müller, is an endemic disease of the wild American hazelnut, *Corylus americana* Marsh. The fungus is found associated with *C. americana* throughout its native range (Barss, 1930; Farr et al., 1989), which spans the eastern U.S., from Maine to Saskatchewan, Canada, south to Georgia and Louisiana, and west to Oklahoma (Gleason and Cronquist, 1998). Although EFB is typically inconsequential to its natural host, *C. americana* (Fuller, 1908; Weschcke, 1954), the disease causes severe cankering, dieback, and death of its European relative, the hazelnut of commerce, *C. avellana* L. (Johnson and Pinkerton, 2002). It is the main reason attempts at commercial production of European hazelnuts failed in the eastern U.S. (Fuller, 1908; Lagerstedt, 1975; Thompson et al., 1996). EFB was discovered in commercial hazelnut orchards in southwest Washington, an area outside of its native range, in the early 1970s (Davison and Davidson, 1973). Since then, it has spread throughout much of the Willamette Valley of Oregon where 99% of the U.S. commercial hazelnut crop is grown (Mehlenbacher, 2003, 2005a). Consequently, EFB is a threat in all regions of North America where hazelnuts are now grown as well as those regions suitable for future production. Therefore, the identification and development of cultivars resistant to the disease is a necessary objective of hazelnut breeding programs in North America.

Over the past 30 years, much of the genetic improvement work in the U.S. has centered on using the 'Gasaway' single dominant allele for resistance to EFB (Mehlenbacher, 2005b; Mehlenbacher and Smith, 2004; Mehlenbacher and Thompson, 1991a, 1991b). Recently, breeding for resistance has been facilitated by the identification of several random amplified polymorphic DNA (RAPD) markers tightly linked to the 'Gasaway' gene (Davis and Mehlenbacher, 1997; Mehlenbacher et al., 2004). Marker-assisted selection is now routinely and effectively used in the hazelnut breeding program at Oregon State University (OSU), Corvallis, Ore., to screen progeny segregating for the 'Gasaway' allele in absence of the fungus (S. Mehlenbacher, personal communication, 2006; Chen et al., 2005; Lunde et al., 2000). Although the 'Gasaway' gene has yet to be overcome by *A. anomala* in the Pacific Northwest, hazelnut breeders have been concerned with the long-term stability of using only one source of single gene resistance (Coyne et al., 1998; Osterbauer, 1996; Pinkerton et al., 1998) and have been searching for additional sources. Fortunately, sources of qualitative and quantitative resistance have been identified in several *C. avellana* cultivars and selections as well as other *Corylus* L. species and interspecific hybrids (Chen, 2003; Chen et al., 2005; Coyne et al., 1998; Coyne et al., 2000; Farris, 2000; Lunde et al., 2000; Pinkerton et al., 1993; Rutter, 1991). Because *C. avellana* generally has the most desirable nut characteristics within the genus (Mehlenbacher, 1990), the identification of genetic resistance to EFB within this species holds promise for more rapid development of EFB-resistant cultivars with high nut yields and acceptable kernel quality. This is because we expect to need fewer backcross generations if the donor resistance belongs to *C. avellana*.

Until recently, germplasm from much of the former Soviet Union was unavailable for evaluation by breeders in North America. In late Aug. 2002, the authors made a germplasm exploration and collection trip to the Russian Federation and the Crimean Peninsula of the Ukraine. Visits were made to the known institutions that have conducted hazelnut research in the Russian Federation: 1) the Forestry Institute of the Timiryazev Agricultural Academy, Moscow; 2) the Research Institute for Horticulture and Viticulture, Krasnodar; 3) the VIR Research Institute of Plant Industry, Krymsk; 4) the VIR Breeding Station, Maykop; 5) and the Russian Academy of Agricultural Sciences Institute of Floriculture and Subtropical Cultures, Sochi; and in the Ukraine, the Nikita Botanical Gardens, Yalta. The expedition resulted in the introduction of a diverse collection of hazelnut germplasm. The purpose of this study was to evaluate a large subset of this new germplasm for response to inoculation with the incitant of EFB, *A. anomala*, in an effort to identify novel sources of genetic resistance to this pathogen.

Materials and Methods

Plant material. Hazelnut germplasm in the form of nuts resulting from open pollination was collected from various locations throughout southern Russia and the Crimean Peninsula, Ukraine, in late Aug. through

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Table 1. Accession data for Russian and Crimean hazelnut (*Corylus avellana* L.) germplasm inoculated with *Anisogramma anomala* (Peck) E. Muller in 2003–2004.

Seed source ID #	Collection source (cultivar if known) and collection location	No. of nuts stratified[z]/no. germ.	No. of seedlings. inoc.[y]
RUS-1	Institute of Floriculture and Subtropical Cultures, hazelnut breeding germplasm collection—many cultivars mixed; Sochi, Russia	920/162	91
RUS-2	Institute of Floriculture and Subtropical Cultures, 'Kudashovski'; Sochi, Russia	125/60	0
RUS-3	Sochi Market #1; Sochi, Krasnodarskiy Kray, Russia	150/25	15
RUS-4	Sochi Market #2; Sochi, Krasnodarskiy Kray, Russia	120/37	18
RUS-5	Sochi Market #3; Sochi, Krasnodarskiy Kray, Russia	140/51	25
RUS-6	Sochi Market #4; Sochi, Krasnodarskiy Kray, Russia	100/39	14
RUS-7	Sochi Market #5; Sochi, Krasnodarskiy Kray, Russia	80/25	10
RUS-8	Sochi Market #6; Sochi, Krasnodarskiy Kray, Russia	154/23	13
RUS-9	Holmskij Market #1; Holmskij, Krasnodarskiy Kray, Russia	69/25	15
RUS-10	Holmskij Market #2; Holmskij, Krasnodarskiy Kray, Russia	107/32	18
RUS-11	Holmskij Market #3; Holmskij, Krasnodarskiy Kray, Russia	129/47	23
RUS-12	Holmskij Market #4; Holmskij, Krasnodarskiy Kray, Russia	100/16	9
RUS-13	Holmskij Market #5; Holmskij, Krasnodarskiy Kray, Russia	71/32	23
RUS-14	Holmskij Market #6; Holmskij, Krasnodarskiy Kray, Russia	123/53	20
RUS-15	VIR Breeding station, hazelnut germplasm collection—many cultivars mixed; Maykop, Krasnodarskiy Kray, Russia	318/157	91
RUS-16	Research Institute of Orchard and Wine Production, 'Badem'; Krasnodar, Krasnodarsky Kray, Russia	100/34	25
RUS-17	Krasnodar Market #1; Krasnodar, Krasnodarskiy Kray, Russia	140/17	8
RUS-18	Krasnodar Market #2; Krasnodar, Krasnodarskiy Kray, Russia	143/38	20
RUS-19	Krasnodar Market #3; Krasnodar, Krasnodarskiy Kray, Russia	126/57	22
RUS-20	Krasnodar Market #4; Krasnodar, Krasnodarskiy Kray, Russia	168/37	20
RUS-21	Simferopol Roadside Market #1A; near Simferopol, Crimea, Ukraine	252/33	17
RUS-22	Simferopol Roadside Market #1B; near Simferopol, Crimea, Ukraine	110/37	17
RUS-23	Simferopol Roadside Market #2; near Simferopol, Crimea, Ukraine	177/38	20
RUS-24	Simferopol Roadside Market #3; near Simferopol, Crimea, Ukraine	220/26	9
RUS-25	Simferopol Roadside Market #4; near Simferopol, Crimea, Ukraine	197/39	17

continued

early Sept. 2002. Nuts were obtained from horticultural institutes and breeding stations in Russia and Crimea as well as purchased from local roadside vendors and markets (Table 1). A total of 4790 nuts representing 32 seed sources were brought to Rutgers University for evaluation in New Jersey (Table 1), and a similar collection of nuts was brought to the hazelnut breeding program at OSU.

The nuts brought to Rutgers University were stratified in moistened peatmoss in polyethylene bags at 4 °C from mid-Sept. 2002 until mid-Jan. 2003. Nuts were then removed from stratification and germinated in flats (43.2 cm × 33.0 cm × 6.4 cm) containing a peat-based planting medium (Promix BX; Premier Horticulture, Rivière-du-Loup, Québec) in a greenhouse maintained at 24 °C day/18 °C night with 16-h daylengths. After ≈4 weeks, 1285 seedlings were transplanted to 3.7-L plastic containers using the same planting medium. Each plant was top-dressed with 5 g of slow-release fertilizer (Osmocote Plus 15N–3.9P–10K with micronutrients 5 to 6 months; The Scotts Co., Marysville, Ohio) and watered as needed.

Greenhouse inoculations. At 6 to 8 weeks after germination (late Feb. to early Mar. 2003), a total of 605 seedlings representing 29 of the 32 seed sources were inoculated with *A. anomala* in the greenhouse. To measure the effectiveness of the inoculations, 33 seedlings from controlled crosses between susceptible parents were included (Table 1). Hazelnut stems containing mature *A. anomala* stromata were collected in Jan. 2003 from infected plants growing at the Rutgers Horticultural Research and Extension Farm 2, North Brunswick, N.J., and the Rutgers Fruit Research and Extension Center, Cream Ridge, N.J., and stored at –20 °C in doubled polyethylene bags until needed. Ascospore suspensions were prepared by dissecting whole stromata from stem pieces, hydrating the stromata in sterile distilled water, then crushing the stromata with a mortar and pestle to release the ascospores (Johnson et al., 1994). The resulting suspension was filtered through two layers of cheesecloth and diluted to $\approx 1 \times 10^6$ ascospores per milliliter in sterile distilled water with the aid of a bright-line hemocytometer (Hausser Scientific Co., Horsham, Pa.). Approximately 150 seedlings were placed in each of two chambers constructed of a PVC tube frame (4.88 m × 1.83 m × 0.85 m) set on top of a greenhouse bench and completely covered with 0.1-mm (4-mil) polyethylene sheeting. Humidifiers (Trion Herrmidifier 707 Series; Herrmidifier Co., Sanford, N.C.) were placed at each end of the chamber and run as needed to maintain relative humidity near 100% for the entire inoculation period. On sunny days, shadecloth and venting of side flaps were used to keep the chamber close to ambient greenhouse temperatures (24 °C day/18 °C night). Ascospore suspensions were applied directly to the newly expanding shoot tips of each seedling by spraying with a handheld pump sprayer. Each seedling was inoculated twice,

Table 1. (*continued*) Accession data for Russian and Crimean hazelnut (*Corylus avellana* L.) germplasm inoculated with *Anisogramma anomala* (Peck) E. Muller in 2003–2004.

Seed source ID #	Collection source (cultivar if known) and collection location	No. of nuts stratified[z]/no. germ.	No. of seedlings. inoc.[y]
RUS-26	Simferopol Roadside Market #5; near Simferopol, Crimea, Ukraine	173/24	11
RUS-27	Dzhubga Market #1, Dzhubga, Russia	55/11	11
RUS-28	Nikita Botanical Gardens #1; Nikita Botanical Gardens, Yalta, Crimea, Ukraine	100/53	18
RUS-29	Nikita Botanical Gardens #2; Nikita Botanical Gardens, Yalta, Crimea, Ukraine	86/34	0
RUS-30	Nikita Botanical Gardens #3; Nikita Botanical Gardens, Yalta, Crimea, Ukraine	6/3	3
RUS-31	Wild *C. avellana*, near Moscow, Russia	17/5	0
RUS-32	Nikita Botanical Gardens #4; Nikita Botanical Gardens, Yalta, Crimea, Ukraine	14/2	2
	Totals	**4790/1285**	**605**
	Susceptible control seedlings		
RF-11	Segorbe x Rutgers H7–39	—	2
RF-17	Casina x Ennis	—	14
VA-08	Butler x Grimo 208D	—	17
		Total	**33**

[z]Number of nuts stratified represents all those brought to Rutgers University.
[y]Seedlings not inoculated with *Anisogramma anomala* were moved to an eastern filbert blight free greenhouse and were planted at the Rutgers Fruit Research and Extension Center, Cream Ridge, N.J., in June 2003.

first on 26 Feb. 2003 and second on 3 Mar. 2003. They remained in the humidity chamber for 7 d after the final inoculation with humidity levels being reduced incrementally over the last 4 d. This was accomplished by gradually decreasing the run time of the humidifiers and by venting the chamber. Once inoculations of the first two groups of seedlings were completed, they were removed from the chambers and a second group of approximately the same number was placed inside each and inoculated following the same protocol on 13 Mar. 2003 and 17 Mar. 2003.

After inoculation, seedlings were maintained in the greenhouse under optimal growing conditions until early June 2003. They were then moved outside under 40% shadecloth for 2 weeks and were planted in the field in late June 2003 (0.76 m between plants by 3.66 m between rows) at the Rutgers University Vegetable Research and Extension Farm, North Brunswick, N.J. Weed control, irrigation, and fertilizer were provided as needed.

Field inoculations. To reduce the chance of susceptible plants escaping infection and to increase disease pressure in the planting, seedlings were ''field'' inoculated before budbreak in Spring 2004. Branches containing mature *A. anomala* stromata were collected in Jan. 2004 from the two previously mentioned collection sites and stored at –20 °C in doubled polyethylene bags until needed. In early Apr. 2004, the branches were removed from the freezer and cut into 10- to 15-cm pieces. The cut ends were then sealed by dipping 2 to 3 cm of each end into a melted mix of equal proportions grafting wax (Trowbridge's Grafting Wax; Walter E. Clark and Sons, Orange, Conn.) and paraffin wax. This was done to conserve moisture in the stick in an attempt to prolong the life of the biotrophic fungus it contained and to extend the release period of the ascospores. Approximately equal amounts of cankered wood were collected from both research farms. After being sealed with wax, both sources of infected wood were combined and mixed together thoroughly before tying to the seedlings to ensure that an even spread of disease from both locations was made. The plants were ''field inoculated'' by tying one diseased stick in the canopy of every seedling, including controls (638 total).

In Dec. 2005, a thorough visual rating of disease severity was recorded for each existing plant using an index similar to that developed by Pinkerton et al. (1992): 0 = no detectable EFB, 1 = single canker, 2 = multiple cankers on single branch, 3 = multiple branches with cankers, 4 = greater than 50% of the branches with cankers, and 5 = all branches containing cankers, excluding basal sprouts. Plants scoring 0 or 1 were considered resistant to infection by *A. anomala*.

Random amplified polymorphic DNA markers linked to the 'Gasaway' gene for eastern filbert blight resistance. Leaf samples from 14 seedlings that expressed no symptoms or signs of EFB in late May 2005 were sent to OSU to undergo DNA extraction and screening for presence of RAPD markers closely linked to the 'Gasaway' gene for EFB resistance. Approximately eight young leaves of each plant were collected in polyethylene bags, packed in a cooler, and shipped overnight to the hazelnut breeding laboratory at OSU. DNA extractions were made on 31 May 2005 and polymerase chain reaction (PCR) assays completed on 10 Aug. 2005 following the protocol of Lunde et al. (2000). DNA was screened with three RAPD primers (UBC 152_{800}, UBC 268_{580}, and OP $AA12_{850}$) routinely used by the OSU hazelnut breeding program to identify seedlings segregating for the 'Gasaway' allele that confers resistance to EFB (relative position to the resistance gene for markers UBC 152_{800}, UBC 268_{580}, and OP $AA12_{850}$ is 1.4, 5.8, and 0.0 cM, respectively) (Chen et al., 2005; Davis and Mehlenbacher, 1997; Mehlenbacher et al., 2004). Amplification products were separated by electrophoresis on 2% agarose, stained with ethidium bromide, destained, visualized with a transilluminator, and then photographed.

Leaf samples of two additional seedlings, which expressed no or very minor signs of the pathogen at the final rating in Dec. 2005, were sent to OSU for screening in July 2006. DNA extractions and PCR assays were completed on 16 July 2006 following the same protocol. However, the two seedlings were limited to RAPD primers UBC 152_{800} and OP $AA12_{850}$.

Results and Discussion

Identification of eastern filbert blight-resistant seedlings. Of the original 605 inoculated hazelnut seedlings, eight showed no signs or symptoms of infection by *A. anomala*. Five additional seedlings showed only very minor signs of the pathogen as each expressed only one small canker (Table 2). Of these 13 seedlings, seven originated from nuts purchased from roadside vendors located near Simferopol, Crimea, Ukraine; five originated from nuts purchased at an outdoor market in the village of Holmskij (near Krasnodar), Russia; and one originated from nuts obtained from the hazelnut breeding program of the Nikita Botanical Gardens, Yalta, Crimea, Ukraine (Tables 1 and 2).

In Dec. 2005, 13.4% (81 of 605) of the plants from the original inoculated collection were no longer suitable for evaluation, primarily as a result of death in 2004 or early 2005 from EFB. Nearly 98% of the remaining population expressed multiple cankers with 89.7% (470 of 524) rating 4 or 5 (Table 3). All 32 of the control seedlings expressed numerous EFB cankers with the EFB severity

Table 2. Results of polymerase chain reaction assay of seedlings showing no or very minor symptoms of eastern filbert blight (EFB) in Dec. 2005 using Random amplified polymorphic DNA (RAPD) primers closely linked to the 'Gasaway' gene for resistance.

Plant number; Seed source (Table 1)	EFB response[z]	Canker length (cm)	No. of stromata[y]	RAPD marker linked to Gasaway allele[x]		
				UBC 268_{580}	UBC 152_{800}	OP $AA12_{850}$
Holmskij market, near Krasnodar, Russia						
H3R13–40; RUS-9	0	0	0	0	0	0
H3R7–25; RUS-12	1	30	>30	N/A[w]	0	0
H3R4–23; RUS-13	0	0	0	0	0	0
H3R4–28; RUS-13	1	20	<5	**1**	0	0
H3R4–30; RUS-13	0	0	0	0	0	0
Simferopol roadside markets, Crimea, Ukraine						
H3R14–26; RUS-22	0	0	0	0	0	0
H3R12–58; RUS-23	0	0	0	0	0	0
H3R12–62; RUS-23	0	0	0	0	0	0
H3R4–5; RUS-25	1	3	0	0	0	0
H3R7–7; RUS-26	0	0	0	N/A[w]	0	0
H3R7–9; RUS-26	1	6	10	0	0	0
H3R7–11; RUS-26	1	8	0	0	0	0
Nikita Botanical Garden, Yalta, Crimea, Ukraine						
H3R10–88; RUS-28	0	0	0	0	0	0

[z]Plants were visually rated using an index as follows: 0 = no detectable EFB, 1 = single canker. Seedlings scoring 0 or 1 are considered highly resistant.
[y]Number of fully formed stromata in *Anisogramma anomala* (Peck) E. Muller canker.
[x]DNA extractions and polymerase chain reaction assays were completed at Oregon State University, Corvallis, Ore. Polymerase chain reaction assay: 0 = marker band absent; 1 = marker band present.
[w]Seedling not included in polymerase chain reaction assay.
N/A, not available.

Table 3. Disease ratings for Russian and Crimean *Corylus avellana* L. and susceptible controls after inoculation with *Anisogramma anomala* (Peck) E. Muller.

Seed source ID	No. of plants living[z]	Disease rating[y]						
		Ave.	0	1	2	3	4	5
RUS-1	77	4.8	0	0	0	2	15	60
RUS-3	15	4.4	0	0	0	0	9	6
RUS-4	15	4.9	0	0	0	0	2	13
RUS-5	22	4.4	0	0	0	3	8	11
RUS-6	14	4.3	0	0	0	2	6	6
RUS-7	10	4.3	0	0	1	2	0	7
RUS-8	11	4.3	0	0	0	0	8	3
RUS-9	9	3.9	1	0	1	0	2	5
RUS-10	16	4.7	0	0	0	1	3	12
RUS-11	15	4.3	0	0	0	1	8	6
RUS-12	8	3.9	0	1	0	1	3	3
RUS-13	17	3.7	2	1	0	0	8	6
RUS-14	15	4.3	0	0	2	1	3	9
RUS-15	84	4.8	0	0	0	6	7	71
RUS-16	22	4.3	0	0	1	2	9	10
RUS-17	7	4.4	0	0	0	0	4	3
RUS-18	19	4.5	0	0	1	1	5	12
RUS-19	20	4.6	0	0	0	1	7	12
RUS-20	19	4.5	0	0	0	1	7	11
RUS-21	16	4.5	0	0	1	1	3	11
RUS-22	16	4.4	1	0	0	1	3	11
RUS-23	16	4.0	2	0	1	0	3	10
RUS-24	8	4.8	0	0	0	1	0	7
RUS-25	15	3.7	0	1	1	4	4	5
RUS-26	8	3.3	1	2	0	0	1	4
RUS-27	10	4.8	0	0	0	1	0	9
RUS-28	16	4.4	1	0	0	0	5	10
RUS-30	2	5.0	0	0	0	0	0	2
RUS-32	2	5.0	0	0	0	0	0	2
Totals	**524**	**4.4**	**8**	**5**	**9**	**32**	**133**	**337**
Susceptible control progeny								
RF-11	2	5.0	0	0	0	0	0	2
RF-17	14	4.9	0	0	0	0	1	13
VA-08	16	4.4	0	0	0	3	6	8
Totals	**32**	**4.8**	**0**	**0**	**0**	**3**	**7**	**23**

[z]Plants available for rating in Dec. 2005.
[y]Plants were visually rated using an index as follows: 0 = no detectable eastern filbert blight, 1 = single canker, 2 = multiple cankers on single branch, 3 = multiple branches with cankers, 4 = greater than 50% of the branches with cankers, 5 = all branches containing cankers, except for basal sprouts. Seedlings scoring 0 or 1 are considered highly resistant. Seedlings scoring 2 are considered moderately resistant.

rating for the control group averaging 4.77 (Table 3). The uniform infection of control seedlings, plus the very high disease incidence and EFB severity rating for the entire population (average 4.37) (Table 3), supports our conclusion that the combination of greenhouse and field inoculations was effective at reducing the likelihood that seedlings identified as symptomless are the result of escape from infection by *A. anomala*. Therefore, it is likely that seedlings expressing no or very minor symptoms of EFB represent sources of resistance to infection by the fungus. The clustering of resistant plants based on their origin (Table 2) gives further evidence that genetic resistance has been identified in the evaluated germplasm collection. Although 13 apparently highly resistant seedlings were identified, it is probable that only two new sources of genetic resistance have been revealed. The clustering of resistance in distinct geographic areas suggests that there may be two unique sources of resistance: one in Simferopol, Ukraine, and the other in Holmskij, Russia. Because Holmskij is more than 400 km from Simferopol, and the hazelnuts collected in the two regions were purchased from roadside vendors and appeared to be local selections (or possibly nuts collected from the wild), it is likely that the two sources of genetic resistance are unrelated. Given that the Nikita Botanical Gardens is located less than 50 km from Simferopol, and local Crimean hazelnut selections are used in their genetic improvement work (Yezhov et al., 2005), it is probable that the Nikita Botanical Gardens resistant seedling contains a similar source of resistance to those from Simferopol.

Random amplified polymorphic DNA markers linked to the 'Gasaway' gene for eastern filbert blight resistance. Of the 14 seedlings that were symptomless in late May 2005, of which leaf samples were sent to OSU for DNA extraction and PCR assay, 11 developed no or very minor signs of the pathogen in Dec. 2005. At this time, two additional seedlings (not of the original 14 assayed) were identified in the collection that also developed no or very minor signs of the pathogen of which leaf samples were collected for DNA extraction and PCR assay in July 2006. All 13 of these apparently resistant seedlings failed to generate RAPD markers for the primers UBC 152_{800} and OP $AA12_{850}$. In addition, none of the resistant seedlings screened with the primer UBC 268_{580}, except seedling H3R4-28, generated a RAPD marker (Table 2). Because RAPD primers occasionally amplify bands of the same or similar size as the marker in susceptible genotypes (S. Mehlenbacher, personal communication, 2006), the presence of the band generated by UBC 268_{580} for seedling H3R4-28 is not conclusive evidence that it is related to 'Gasaway'. Although the results for this seedling were inconclusive, the RAPD marker data for the remaining 12 strongly support that they represent sources of genetic resistance unrelated to 'Gasaway' (Chen et al., 2005; Davis and Mehlenbacher, 1997; Lunde et al., 2000; Mehlenbacher et al., 2004). Further linkage studies using additional RAPD markers or other molecular fingerprinting techniques such as the recently developed microsatellite marker system for hazelnuts (Bassil et al., 2005; Boccacci et al., 2005) would reinforce this claim and help to determine the overall genetic diversity present in the newly identified sources of resistance.

Conclusions and future directions. This study yielded new, potentially very useful, sources of EFB resistance from a previously untested, diverse population of *C. avellana*. These results, in combination with other sources of EFB resistance recently identified in a small number of *C. avellana* cultivars and selections from Spain, Finland, Ukraine, and other locations (Chen, 2003; Lunde et al., 2000; Mehlenbacher, 2005b), support our reasoning that continual germplasm collection and evaluation will likely lead to the identification of additional novel sources of genetic resistance in the species. Although high genetic resistance has been found in other *Corylus* species, finding resistance in a genotype that is high yielding and also has superior nut quality is rare. Discovering this combination in an existing genotype will be very beneficial to breeding well-adapted cultivars for areas where the disease is present, conceivably taking years off of the time needed to develop commercially acceptable cultivars. Breeding work using 'Gasaway', an obsolete *C. avellana* pollenizer with low nut yields and very poor nut quality (Mehlenbacher and Thompson, 1991a), as the source of resistance has taken nearly 30 years to develop a commercially acceptable cultivar for the kernel market (Mehlenbacher, 2005b).

The stability of the new sources of genetic resistance identified in this study will be evaluated over the next several years as they are challenged with a more diverse collection of *A. anomala* isolates. In addition to the seedlings assigned a score of 0 or 1, seedlings assigned a score of 2 (multiple cankers on a single branch) have been retained for further evaluation. Interestingly, of the nine seedlings assigned rating of 2, three originated from Holmskij and three from Simferopol (Table 3), adding further evidence of genetic resistance from these locations. Plants receiving this score, although they expressed multiple cankers, appeared to grow relatively normal as they showed little to no dieback with stem tissue distal to the cankers still living. The three remaining seedlings that scored similarly, one from Sochi and two from Krasnodar (Table 3), may represent additional sources of moderate resistance that may prove to be of use to the genetic improvement program (although the seedlings from Krasnodar may be related to those from Holmskij as a result of their close proximity).

In addition to longer-term response to EFB, seedlings will simultaneously be evaluated for characteristics such as nut yield and quality (size, percent kernel, blanching, and flavor), cold hardiness, flowering dates, incompatibility alleles, bud mite (*Phytoptus avellanae* Nal.) resistance, growth habit, and so on. The mode of inheritance of resistance of the most promising selections will also be investigated. The top performers will be assessed for use in the Rutgers and OSU hazelnut genetic improvement program and be made available to the U.S. Department of Agriculture, Agricultural Research Service National Clonal Germplasm Repository in Corvallis, Ore.

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