

Hazelnut Accessions Provide New Sources of Resistance to Eastern Filbert Blight

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Abstract. A diverse collection of 58 hazelnut accessions, including *Corylus avellana* L. and interspecific hybrids, were evaluated for their response to the eastern filbert blight pathogen *Anisogramma anomala* (Peck) E. Müller after greenhouse inoculation. Evaluations were made using enzyme-linked immunosorbent assay and visual inspection. Forty-five of these became infected, 12 remained free of infection, and one gave inconclusive results. The 12 accessions showing complete resistance were European hazelnuts ‘Culpla’ from Spain and CCOR 187 from Finland; *C. americana* × *C. avellana* hybrids ‘G081S’, CCOR 506, and Weschcke selections TP1, TP2 and TP3; *C. colurna* × *C. avellana* hybrids Chinese Trazels Gellatly #6 and #11; Turkish Trazel Gellatly #3 and backcross hybrid ‘Lisa’; and *C. heterophylla* var. *sutchuensis* × *C. avellana* hybrid ‘Estrella #1’. In a second test, exposure of potted trees under structures topped with diseased wood confirmed the complete resistance of ‘Santiam’, four pollinizers, and ‘Ratoli’. However, a few small cankers were observed on ‘Closca Molla’ from Spain and OSU 729.012, with resistance from *C. californica* (A.DC.) Rose, in contrast to the results of earlier greenhouse inoculations.

The 12,000 ha of the European hazelnut, *Corylus avellana* L., in Oregon’s Willamette Valley produce 98% of the United States crop and 3% to 5% of the world crop (FAO Production Yearbook, 2003). The Oregon hazelnut industry is seriously threatened by eastern filbert blight (EFB) incited by the pyrenomycete *Anisogramma anomala* (Peck) E. Müller. The fungus is endemic on the American hazelnut (*C. americana* Mill.) in eastern North America. On susceptible European cultivars, it causes severe cankers, rapid yield loss, and eventually tree death in 5 to 12 years if control measures are not practiced (Pinkerton et al., 1993). Control practices include pruning of infected branches and fungicide applications. However, because of the expense of fungicide applications and the dramatic yield loss incited by severe pruning

of cankers, genetic resistance is the most desirable and economic means of disease control (Mehlenbacher, 1994). Therefore, developing cultivars resistant to EFB is a goal of the Oregon State University (OSU) hazelnut breeding program.

Complete resistance to EFB was first discovered in the obsolete pollinizer ‘Gasaway’ (Cameron, 1976). Genetic studies showed that complete resistance is conferred by a single dominant gene (Mehlenbacher et al., 1991). ‘Gasaway’ has been the major source of resistance used in the OSU breeding program. However, ‘Gasaway’ has low yields and undesirable nut and kernel characteristics, thus requiring considerable effort to combine the resistance gene with the many attributes required of a commercially acceptable cultivar. Furthermore, concern exists about the durability of a single resistance gene because a new race of *A. anomala* could potentially overcome it (Johnson et al., 1996). The identification of additional sources of genetic resistance would be desirable.

Inoculation of European hazelnut cultivars with the EFB pathogen has revealed additional sources of complete resistance. ‘Zimmerman’, an uninfected tree identified in a hedgerow near a severely infected orchard near Boring, Ore. (Pinkerton, pers. comm.), remained free of disease after greenhouse inoculations (Coyne, 1995). ‘Closca Molla’ and ‘Ratoli’, both superior in many horticultural respects to ‘Gasaway’, displayed no symptoms of EFB after greenhouse inoculations (Lunde et al., 2000). Complete resistance has also been detected in numerous accessions of *Corylus* species and interspecific hybrids (Coyne et al., 1998; Lunde et al., 2000).

The fungus *A. anomala* has a 2-year life cycle that includes an incubation period of 12 to 14 months before symptoms are expressed (Gottwald and Cameron, 1980; Johnson et al., 1994; Pinkerton et al., 1995). Thus, evaluation by observing canker development on the field is a slow process. An indirect enzyme-linked immunosorbent assay (ELISA) after greenhouse inoculation shortens the detection time to 6 months and offers a reliable method for evaluation of genotypes for complete resistance (Coyne et al., 1996).

In this study, 58 hazelnut accessions from the collections of the OSU hazelnut breeding program and the U.S. Department of Agriculture, Agricultural Research Service National Clonal Germplasm Repository in Corvallis, Ore., were evaluated for response to EFB inoculation. In a second study, we exposed potted trees to an infection source to quantify susceptibility of genotypes that had been reported as completely resistant during earlier greenhouse tests.

Materials and Methods

Scions of 58 accessions were collected in Dec. 2000 and stored at 0 °C. Three scions per accession were grafted onto *C. avellana* rooted layers in spring 2001. Grafted trees were planted in 5-L pots containing a mix of equal volumes of peat, pumice, fine bark dust, and 9 g Sierra 3- to 4-month release fertilizer (18N–2.6P–9.96K) (Scotts Co., Marysville, Ohio). The grafted trees were kept in the greenhouse under optimal conditions for growth (24 °C day/18 °C night) until they were ready for inoculation, usually 3 to 4 weeks later. ‘Gasaway’ was included as a resistant control and ‘Ennis’ or ‘Daviana’ as susceptible controls.

Two inoculation chambers were set up in the greenhouse using PVC tubing (1.27-cm diameter) placed on top of benches (1.22 × 0.44 m) and covered with white 4-mil (0.10-mm) polyethylene sheeting. A humidifier was placed in each inoculation chamber and was programmed to run from 12:00 noon to 6:00 PM and from 12:00 midnight to 4:00 AM. Plants were inoculated when shoots had four to five nodes (Coyne et al., 1996). Diseased twigs with mature stromata were collected from trees at Oregon State University’s North Willamette Research and Extension Center (NWREC), Aurora, in Nov. 2000 and 2001, and were stored at –20 °C in polyethylene bags until used as inoculum. Perithecia were dissected from the stromata of diseased twigs and ground with a mortar and pestle to release ascospores. The ascospore suspensions were then diluted in distilled water to 1 × 10⁶ spores/mL. The suspensions contained in a squeeze bottle were sprayed to the tips of one or two actively growing shoots on each tree. The sites of inoculation were indicated by tape placed two to three nodes below the apical meristem. The inoculations were repeated three times at 3-d intervals. After inoculation, the trees remained in the greenhouse under optimal growing conditions for 6 months before the infection assay. One

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replication of each greenhouse-inoculated accession was tested to score for the presence of the fungus 6 months after inoculation using the ELISA of Coyne et al. (1996), as slightly modified by Lunde et al. (2000). If the first tree gave a positive ELISA reading, the other two trees of that accession were transported to the field at NWREC in fall 2001 and planted in a nursery row, and cankers were measured in Jan. 2003. If the first tree showed a negative or inconclusive result, the other two trees were tested by ELISA a few weeks later. Accessions that were free of infection after the first year were reinoculated in spring 2002, when new shoots had grown out, and were re-assayed 6 months later. An accession was scored as susceptible if one or more of the three trees was scored as positive by ELISA or if symptoms were observed. An accession was scored as completely resistant if all three trees were scored as negative by ELISA and no symptoms were observed for 2 years.

The relative susceptibility of 120 hazelnut genotypes, most of which were numbered *C. avellana* selections from the breeding program, was quantified by exposing potted trees, generally a dozen of each, under structures topped with EFB-diseased wood. The method has been described by Mehlenbacher et al. (2001) and is based on that of Pinkerton et al. (1993). The trees were placed under the structure in randomized positions, with equal numbers in each of four blocks. Trees were exposed in Spring 2004, and cankers were counted and measured in early Jan. 2006. Canker lengths were summed for each tree, a square root transformation was used to remove the association between mean and variance, and mean canker lengths provided a ranking of relative susceptibility of genotypes. Based on the results of earlier tests, we included five control cultivars: Daviana (highly susceptible), Barcelona (intermediate), Willamette (moderately resistant), Lewis (moderately resistant), and Tonda di Giffoni (high quantitative resistance). Trees of selection OSU 729.012, 'Ratoli', 'Closca Molla', and 'Santiam', and four recently released pollinizer cultivars (Gamma, Delta, Epsilon, and Zeta) were included. All eight had shown complete resistance in earlier greenhouse tests (Lunde et al., 2000, and unpublished data). OSU 729.012 is from a cross of VR33-17 [*C. californica* M-9 × OSU 14.084 ('Barcelona' × 'Daviana')] and OSU 350.089 ('Tombul Ghiaghli' × 'Tonda Romana'), with resistance derived from the wild grandparent M-9. Ratoli and Closca Molla are minor Spanish cultivars. 'Santiam', 'Gamma', and 'Delta' have resistance from 'Gasaway', whereas 'Epsilon' and 'Zeta' have resistance from 'Zimmerman'. Fewer than a dozen trees (range, three to eight trees) were included of genotypes with resistance from 'Gasaway' or 'Zimmerman' (Table 1).

Results and Discussion

Using the ELISA method, the response of hazelnut accessions to EFB was separated

Table 1. Results of exposing potted hazelnut trees to eastern filbert blight in spring 2004.

Cultivar	No. of trees ^z		Canker length ^y	No. of cankers ^x
	Exposed	Infected		
Daviana	12	12	13.05	7.6
Barcelona	12	12	10.36	5.2
Willamette	10	10	7.83	4.7
Lewis	12	11	6.60	3.4
Tonda di Giffoni	12	9	3.76	1.3
Closca Molla	8	5	2.82	1.4
OSU 729.012	10	6	2.41	1.3
Ratoli	11	0	0.00	0.0
Santiam	7	0	0.00	0.0
Gamma	3	0	0.00	0.0
Delta	8	0	0.00	0.0
Epsilon	3	0	0.00	0.0
Zeta	3	0	0.00	0.0
LSD _{0.05}	—	—	2.03	1.8

^zNumber of potted trees exposed and number infected with eastern filbert blight. For most genotypes in the test, 12 potted trees were exposed.

^yMean of total canker length per tree, expressed on a square root scale. Cankers were measured on 5, 6, and 10 Jan. 2006.

^xMean number of cankers per tree.

^wLSD values were calculated based on 102 susceptible *Corylus avellana* genotypes (data not shown). Selections are ranked from most to least disease based on total canker length per tree, expressed on a square root scale.

into two distinct categories—completely resistant (Table 2) or susceptible (Table 3)—except for Chinese Trazel Gellatly #4, which showed inconclusive results. For the trees moved to the field at NWREC, 88% of them developed cankers of various lengths after 16 to 18 months (Table 3). The negative control 'Gasaway' and the positive controls 'Ennis' and 'Daviana' behaved as expected. A total of 12 accessions showed complete resistance to *A. anomala* after the greenhouse inoculations (Table 2). They included two *C. avellana* accessions and different types of interspecific hybrids.

Two accessions of *C. avellana*, 'Culpla' and CCOR 187, remained free of infection. 'Culpla' originated in Spain and is similar in appearance to 'Closca Molla'. Its nuts are round and medium size, but more oblate than those of 'Closca Molla'. In Spain, nut yields are moderate to high, nuts are 50% kernel by weight, and are borne in husks the same length as the nuts (Tasias-Valls, 1975). In addition, it is resistant to bud mite (*Phytoptus avellanae* Nal.). CCOR 187 from Finland produces small, round nuts. Unfortunately, this accession will be difficult to use in breeding, because it sets very few female flowers and nuts, and is male sterile (S. Mehlenbacher, pers. comm.).

Five *Corylus americana* × *C. avellana* hybrids showed complete resistance. All five trace to the work of Carl Weschcke in the middle of the 20th century (Weschcke, 1954). He used *C. americana* selections from Wisconsin as parents in breeding. The American hazelnut is the native host of the fungus *A. anomala*. Infection by the fungus results in small cankers on susceptible genotypes of the American hazelnut, but infected areas are walled off in resistant genotypes (Weschcke, 1954). Although the mode of inheritance remains unclear, several completely resistant interspecific hybrids have been reported (Coyne et al., 1998; Lunde et al., 2000; Ourecky and Slate, 1969; Rutter, 1991).

CCOR 507 and G081S are selections of Phil Rutter (Badgersett Research Farm, Canton, Minn.), who began his hybrid hazelnut project using seeds from Weschcke hybrids. Weschcke TP1, Weschcke TP2, and Weschcke TP3 were received as scions from Tom Plocher, who collected them at Carl Weschcke's farm in Wisconsin. The five hybrids are quite variable in nut size, shape, and productivity. Weschcke TP1 has the largest nuts, but also the thickest shells. Weschcke TP2 produces a heavy crop of medium-size nuts with a round, compressed shape and thin shells, and thus appears more promising for use in breeding. CCOR 507, G081S, and Weschcke TP3 produce moderate crops of round nuts. Similar to Yoder #5 (Lunde et al., 2000), Weschcke TP3 is highly

Table 2. Hazelnut accessions resistant to *Anisogramma anomala* after greenhouse inoculation and their origins.

Accession	CCOR ^z	Origin
<i>C. avellana</i>		
Culpla	255	Spain
CCOR 187	187	Finland
<i>C. americana</i> hybrids		
CCOR 507	507	Minn., US
G081S	—	Minn., US
Weschcke TP1	—	Wisc., US
Weschcke TP2	—	Wisc., US
Weschcke TP3	561	Wisc., US
<i>C. colurna</i> hybrids		
Chinese Trazel Gellatly #6	138	B.C., Canada
Chinese Trazel Gellatly #11	173	B.C., Canada
Turkish Trazel Gellatly #3	407	B.C., Canada
Lisa	—	Mich., US
<i>C. heterophylla</i> var. <i>sutchuensis</i> × <i>C. avellana</i> 'Holder'		
Estrella #1	139	Mich., US

^zCorvallis *Corylus* (CCOR) accession number assigned by the U.S. Department of Agriculture, Agricultural Research Service National Clonal Germplasm Repository, Corvallis, Ore.

Table 3. Hazelnut accessions infected by *Anisogramma anomala* after greenhouse inoculations as indicated by a positive enzyme-linked immunosorbent assay and development of cankers in the nursery.

Accession	CCOR ^z	Origin	Canker length (cm) ^y
<i>C. avellana</i>			
AL55	625	Albania	—
Arneson's rootstock	182	Ore., U.S.	14, 0
Blumberger	205	Germany	0, 0
<i>C. avellana</i> 76-1824	—	Armenia ^x	14, 0
Camponica	40	Italy	15, 16
Carrello	376	Italy	—
CCOR 626	626	Albania	8, 0
CCOR 627	627	Sweden	24, 2
Corabel (Fercoril)	482	France	15, 12
Frango #4	660	Poland	—
Frango #5	661	Poland	43, 10
G114S	—	Minn., U.S.	—
Goc	662	Poland	0, 0
Karol	663	Poland	30, 20
Kruse	25	US	30, 7
Locale di Piazza Armerina	371	Italy	10, 7
Maria	668	Poland	6, 0
Nonpareil	37	Ore., U.S.	4, 22
Nostrale	335	Italy	0, 3
Not Tonda Gentile Romana ^w	—	Italy	18, 25
Pellicle Rouge	38	France	3, 0
Pinyolenc	339	Spain	0, 30
Romisondo G1	—	Italy	3, 3
Rosset de Valls	379	Spain	0, 0
Royal	77	Ore., U.S.	7, 5
Tonda di Giffoni	22	Italy	7, 3
Not Volle Zellernuss ^x	315	Germany	0, 0
Warsaw Red	181	Poland	—
Woodford	12	Ore., U.S.	7, 0
<i>C. colurna</i> hybrids			
Chinoka	199	B.C., Canada	17, 33
Chinese Trazel Jemtegaard #1	170	Ore., U.S.	2, 0
Chinese Trazel Jemtegaard #2	164	Ore., U.S.	10, 6
Eastoka	148	B.C., Canada	35, 27
Erioka	201	B.C., Canada	—
Faroka	405	B.C., Canada	0, 0
Filcorn	53	Ore., U.S.	—
Freeoka	154	B.C., Canada	5, 60
Karloka	406	B.C., Canada	23, 17
Laroka	57	B.C., Canada	—
Morrisoka	33	B.C., Canada	0, 7
Turkish Trazel Gellatly #15	408	B.C., Canada	19, 40
Turkish Trazel Gellatly #2	200	B.C., Canada	8, 24
Turkish Trazel Gellatly #5	169	B.C., Canada	4, 0
Zeroka	409	B.C., Canada	16, 30
<i>C. heterophylla</i> var <i>sutchuensis</i> × <i>C. avellana</i> 'Holder'			
Estrella #2	140	Mich., U.S.	—

^zCorvallis *Corylus* (CCOR) accession number assigned by the U.S. Department of Agriculture, Agricultural Research Service National Clonal Germplasm Repository, Corvallis, Ore.

^yTotal length of cankers (in centimeters) for two trees per genotype observed in a nursery row at North Willamette Research and Extension Center. Dashes indicate accessions for which no trees were placed in the nursery.

^xScions obtained from the Mary Flagler Cary Arboretum, New York Botanical Garden, Millbrook, N.Y. 12545.

^wTwo accessions in the collection were not true to name.

susceptible to bud mite. This susceptibility may have been contributed by its *C. americana* parent. Ourecky and Slate (1969) found that 'Rush', a *C. americana* cultivar frequently used in breeding, transmitted its high susceptibility to bud mite to most of its seedlings.

Three selections (Chinese Trazel Gellatly #6, Chinese Trazel Gellatly #11, and Turkish Trazel Gellatly #3) that appear to be first-generation hybrids between the Turkish tree hazel (*C. colurna* L.) and *C. avellana* showed complete resistance. These selections were collected by H.B. Lagerstedt at J.U.

Gellatly's farm in Westbank, B.C., Canada. Despite their names, the "Chinese Trazels" appear to be seedlings of *C. colurna* rather than the Chinese tree hazel, *C. chinensis* Franch. The Turkish tree hazel was used by J.U. Gellatly beginning in the early 1950s to combine the hardiness and nonsuckering growth habit of the tree hazel with the nut size of the European hazel, and the hybrids were named "trazels" (Gellatly, 1956, 1966). 'Lisa', believed to be from the first backcross to *C. avellana* (Farris, 1990; Lukasiewicz, 1992), also showed complete resistance. The four EFB-resistant selections are highly resis-

tant to bud mite, which is consistent with reports that the tree hazel and its interspecific offspring are highly resistant (Farris, 1988). Chinese Trazel Gellatly #6 and Chinese Trazel Gellatly #11 have acceptable nuts and kernels, and have been used as parents in breeding. Results for another selection, Chinese Trazel Gellatly #4, were inconclusive. Of six samples evaluated by ELISA, three values for this accession were just above, and the other three just below, the thresholds. Inoculated trees displayed no symptoms of infection after 18 months in the field. The disease response of Chinese Trazel Gellatly #4 should be investigated further. The phenotype of 'Lisa' (Farris 89AR), selected by Cecil Farris in 1989 from seedlings obtained through the open pollination of 'Grand Traverse' (Lukasiewicz, 1992), suggests that it resulted from a cross of 'Grand Traverse' and *C. avellana*. Lunde et al. (2000) showed that 'Grand Traverse' is completely resistant to EFB. 'Lisa' has attractive features such as good flavor, thin shells, smooth kernels, precocity, and resistance to bud mite (Farris, 1990), but the husks and nuts are long.

The interspecific hybrids designated "Estrella" were obtained by Farris from a cross of *C. heterophylla* var. *sutchuensis* with *C. avellana* 'Holder' (Farris, 1982). Estrella #1 showed complete resistance. Estrella #1 yields well, and the medium-size nuts have a slightly long shape, but it is male sterile (Farris, 1976). In the current study, four out of five Estrella #2 trees evaluated by ELISA showed no infection, but cankers were later observed on inoculated trees. Therefore, we classified it as susceptible to EFB. Estrella #2 is fully fertile, early maturing, and has nut size and shape about equal to its parent 'Holder' (Farris, 1976, 1982).

In the second test, mean canker lengths ranked the control cultivars in the expected order (Table 1). Trees of 'Santiam', the four pollinizers, and 'Ratoli' remained free of disease. However, 5 of 8 trees of 'Closca Molla' and 6 of 10 trees of OSU 729.012 showed small cankers with only a few pustules. The presence of cankers on these two genotypes indicated that they had a lower level of resistance than the six entries listed earlier. The level of quantitative resistance was apparently sufficient for greenhouse-inoculated trees to remain free of infection in two tests (Lunde et al., 2000). These results also indicate that exposure of potted trees to the pathogen should be routinely used to confirm resistance initially detected by greenhouse inoculation and ELISA.

According to Simmonds (1983), if dominant alleles for complete disease resistance are used in a breeding program, the durability of the resistance may be limited. The single dominant allele from 'Gasaway' conferring complete resistance to EFB has been the major source used in the OSU hazelnut breeding program. Additional sources of resistance would be desirable, and in the current study we identified several additional sources of complete resistance to EFB. All the

newly identified resistant *C. avellana* cultivars and interspecific hybrids can be readily crossed with commercial European cultivars.

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