Segregation for Resistance to Eastern Filbert Blight in Progeny of ‘Zimmerman’ Hazelnut

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ABSTRACT. Eastern filbert blight (EFB), caused by the fungus *Anisogramma anomala* (Peck) E. Müller, is an important disease of European hazelnut (*Corylus avellana* L.) in the Pacific northwestern United States. In 1989, a chance seedling free of EFB was discovered adjacent to a severely diseased orchard near Troutdale, Ore. This selection, subsequently named ‘Zimmerman’, was crossed with three susceptible selections. Based on morphological characters and incompatibility alleles, we speculated that ‘Zimmerman’ (S_1S_3) was a hybrid between ‘Barcelona’ (S_1S_2) and ‘Gasaway’ (S_3S_{26}). The three seedling populations were inoculated with spores of the pathogen in a greenhouse test and assayed by indirect enzyme-linked immunosorbent assay (ELISA) and by observation of canker incidence. The observed segregation fit a 3 resistant : 1 susceptible ratio in all three progenies, in contrast to the 1 : 1 ratio found when the resistant pollinizer ‘Gasaway’ was crossed to susceptible genotypes. Random amplified polymorphic DNA (RAPD) marker UBC 152₈₀₀ linked to the resistance gene in ‘Gasaway’ co-segregated with the resistant phenotype in all three populations with 2%, 4%, and 6% recombination, respectively. Seed germination and transplanting records did not provide evidence of selection in favor of resistant seedlings. Pollen germination was 71% in ‘Gasaway’, 29% in ‘Zimmerman’, and 18% in ‘Barcelona’, indicating possible selection at the gametophytic level. Subsequently 16 resistant seedlings of ‘Zimmerman’ were crossed with the highly susceptible selection OSU 313.078. Segregation fit a 3 : 1 ratio in 14 of the 16 progenies, and showed a surplus of resistant seedlings in the other two. None showed a 1 : 1 segregation. Resistance co-segregated with two RAPD markers that flank the ‘Gasaway’ resistance allele. To test allelism of resistance from ‘Gasaway’ and ‘Zimmerman’, VR 6-28 with resistance from ‘Gasaway’ was crossed with ‘Zimmerman’. Eight resistant selections from this progeny were crossed with OSU 313.078. Five of the eight progenies segregated 3 : 1, two progenies segregated 1 : 1, and OSU 313.078 × OSU 720.056 gave only resistant offspring. The ratios indicate that OSU 720.056 is homozygous resistant and that ‘Zimmerman’ and ‘Gasaway’ share a common resistance allele. Reciprocal translocations have been reported in hazelnut cultivars, including ‘Barcelona’, the leading cultivar in Oregon. ‘Zimmerman’ appears to be a hybrid of ‘Barcelona’ and ‘Gasaway’, but because of cytogenetic abnormalities, ‘Zimmerman’ may have inherited two copies of the chromosome region that contain the resistance locus and flanking RAPD markers. If the region containing the resistance were attached to two independent centromeres, a 3 : 1 segregation ratio for disease response and flanking markers would be expected, and we propose this as the most likely explanation. Resistance from ‘Gasaway’ and ‘Zimmerman’ has been called “immunity” or “complete resistance.” However, we noted a few seedlings with small cankers, nearly all of which lacked sporulating stromata. Flanking RAPD markers indicate that the resistance allele is present in these seedlings. Although not “immune” or “completely resistant,” ‘Gasaway’ and ‘Zimmerman’ transmit a very high level of resistance.

Oregon is the top producer of hazelnuts in the United States (Mehlenbacher and Olsen, 1997) and the industry is under threat by the fungus *Anisogramma anomala*, which causes the perennial canker disease eastern filbert blight. This disease is detrimental to production (Johnson et al., 1996) and is now present throughout the Willamette Valley. Host genetic resistance is a desirable way to avoid the expense and time commitment involved in scouting, pruning, and spraying to control this disease (Mehlenbacher, 1995).

Most resistant selections in the hazelnut breeding program at Oregon State University (OSU) derive their resistance from a single source, the obsolete pollinizer ‘Gasaway’. Resistance

from ‘Gasaway’ is conferred by a dominant allele at a single locus (Coyne et al., 1998; Mehlenbacher et al., 1991). It would be beneficial to find new sources of genetic resistance. There is concern that the release of cultivars carrying the ‘Gasaway’ resistance will increase selection pressure on the pathogen, causing it to shift virulence (Pinkerton et al., 1998). A similar scenario occurred in apple (*Malus ×domestica* Borkh.), in which the scab pathogen [*Venturia inaequalis* (Cooke) Aderh.] overcame resistance conferred by V_m (Williams and Brown, 1968) and V_f (Fischer et al., 1994; Parisi et al., 1993).

Davis and Mehlenbacher (1997) used bulked segregant analysis to find five RAPD markers linked to resistance in populations segregating for the ‘Gasaway’ allele. One of these, UBC 152₈₀₀, has proven easy to score and useful for marker-assisted selection in progenies segregating for resistance (Mehlenbacher et al., 2004). Additional RAPD markers were subsequently reported (Mehlenbacher et al., 2004, 2006). Two markers that flank the resistance allele, UBC 152₈₀₀ and UBC 268₅₈₀, are currently used in marker-assisted selection.

In 1989, J. Pinkerton, a plant pathologist at the Horticultural Crops Research Lab of the United States Department of Agriculture, Agricultural Research Service, in Corvallis, Ore., found an

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uninfected chance seedling growing next to a severely infested orchard near Troutdale, Ore. The orchard was owned by the Zimmerman family so the seedling was named 'Zimmerman' (J. Pinkerton, personal communication). Scions of 'Zimmerman' were collected, grafted, and inoculated with the pathogen several times in a severe greenhouse test as described by Coyne et al. (1996). The plants produced neither signs of the pathogen nor symptoms of EFB. The reaction to inoculation is identical to that seen in 'Gasaway', and since they share a common incompatibility allele (S_3), it seemed likely that 'Zimmerman' derived its resistance from 'Gasaway'. We generated several sets of progenies to clarify the inheritance of resistance from 'Zimmerman' and in this paper report the disease responses and RAPD marker data.

Materials and Methods

PLANT MATERIALS. In Feb. 1992, three seedling populations were created using three different advanced selections from the breeding program as seed parents with 'Zimmerman' as the pollen parent in controlled hybridizations, using standard techniques (Thompson et al., 1996). The three seed parents are susceptible to EFB and have diverse pedigrees (Fig. 1). Since progeny of 'Gasaway' segregate 1 resistant:1 susceptible (Mehlenbacher et al., 1991), a similar ratio was expected in the three 'Zimmerman' populations. In 1993, resistant selection VR 6-28 ('Riccia di Talanico' x 'Gasaway') was crossed with 'Zimmerman' to investigate allelism of the two sources of resistance. In 2000, eight selections from this progeny were crossed to highly susceptible selection OSU 313.078, which is from a cross of OSU 23.017 ('Barcelona' x 'Extra Ghiaghli') x 'Tonda Gentile delle Langhe'. Additionally, 16 resistant seedlings from crosses of two susceptible selections, OSU 342.019 and OSU 350.089, with 'Zimmerman' were crossed with OSU 313.078 in 2000 to further investigate segregation ratios.

Nuts were harvested in August from pollinated branches, held in a cool room at 4 °C for 2 months, soaked in water for 4 d, and then stratified in moist vermiculite at 4 °C for about 4 months. Nuts that failed to germinate were partially cracked by hand and treated with 50 mg·L⁻¹ gibberellic acid to break dormancy. After germination, they were planted in 64-cell flats (44.13 x 44.13 cm) and later potted in 3.8- or 5-L pots in a mix containing equal volumes of peat, pumice, and fine douglas fir [*Pseudotsuga menziesii* (Mirbel) Franco] bark dust. Later, 9 g of Sierra 3-4 month release fertilizer (17N-2.6P-9.96K with micronutrients; The Scotts Co., Marysville, Ohio) was added to each pot. Supplemental fertilizer treatments with Peters formula (20N-8.7P-16.6K; The Scotts Co.) were made as needed. Plants were grown in a glasshouse under optimal conditions (24 °C day/18 °C night) during the summer months. Seedlings from 1992 crosses were hardened outdoors in Sept. 1993, and planted in the field in Oct. 1993 at a spacing of 0.92 m in rows spaced 3.05 m apart.

In Dec. 1995, scions were collected from seedlings planted in 1993. Scions were stored at 0 °C until grafted onto *C. avellana* rootstocks in Spring 1996. The grafted trees, three of each seedling, were potted as described above and kept in a glasshouse (24 °C day/18 °C night) with optimal watering until ready for inoculation. Scions were collected in Dec. 1997 from trees that had been scored as resistant in the first test and grafted, inoculated, and assayed in 1998 in order to confirm their phenotype. After inoculation, the trees remained in the glasshouse (24 °C day/18 °C night) with optimal watering for 6 months prior to beginning infection assays.

Fig. 1. Pedigrees of three hazelnut progenies.

GREENHOUSE INOCULATIONS. Twigs with mature stromata were collected from diseased orchards each November–December and stored at –20 °C in polyethylene bags. Spores of the pathogen were dissected from mature perithecia and inoculum consisted of 10⁶ spores/mL suspended in distilled water. Inoculations began once four to five nodes had emerged as described by Coyne et al. (1996). Actively growing shoot tips, usually two to three per grafted tree, were marked with tape three or four nodes below the apical meristem to indicate the place of inoculation. Chamber frames were constructed of polyvinyl chloride tubing (1.27 cm diameter) placed on top of a bench (1.22 x 3.44 m) and covered with white 0.10-mm-thick polyethylene sheeting. About 75 trees were placed in each of two inoculation chambers. A humidifier programmed to run from 1200 to 1800 HR and from 0000 to 0400 HR was placed in each chamber and the plastic was fastened with clothespins. Five days later, the chambers were opened for 2 d. Inoculations were then repeated; all trees received a total of three inoculations.

INFECTION ASSAYS FOR GREENHOUSE-INOCULATED TREES. *Anisogramma anomala* was detected in susceptible genotypes using three assays. ELISA was performed on inoculated shoots of each of two grafted trees from each original seedling as described in Coyne et al. (1996) with the exception that Nunc Maxisorp microtiter plates (catalog no. 439454; Nalge Nunc International, Rochester, N.Y.) were used instead of Corning plates (catalog no. 25860; Corning Glass Works, Corning, N.Y.). The third inoculated tree of each genotype was transported to the Southwest Washington Experiment Station in Vancouver, Wash., where they were planted in a nursery row in Feb. 1997. Canker incidence was evaluated in Feb. and June 1998. For trees inoculated in Winter 1998, microscope sections were stained with 0.05% trypan blue in lactophenol and observed with a light microscope as described by Stone et al. (1992). Sections were scanned for hyphae in samples that gave conflicting results in other assays. Trees that showed any infection by fungal hyphae were scored as susceptible.

FIELD INOCULATIONS. Seedlings from crosses made in 2000 were grown in a glasshouse under optimal conditions (24 °C day/18 °C night) during the summer months as described above. The seedlings were moved outdoors to harden in Sept. 2001 and the following spring were transported to OSU's North Willamette

Research and Extension Center near Aurora, Ore. The potted trees were placed under structures topped with diseased wood (Mehlenbacher et al., 2001; Pinkerton et al., 1993). The structure was equipped with an irrigation system and a timer. When wet, stomata in cankers in the diseased wood emit spores that rain down on the trees below. After 3 months of exposure, the potted trees were lined out in a nursery row. The presence of cankers was noted in Dec. 2004. Trees with very small cankers were carefully inspected for the presence of stomata.

DNA EXTRACTION AND RAPD SCREENING. DNA samples were collected from the original, field-planted seedlings from crosses made in 1993. A very young apical meristem and first leaf were sampled from each tree, and samples were rushed to the laboratory and quickly ground. The method of Lunde et al. (2000), modified from that of Davis et al. (1998), was used with no RNase treatment. When samples were needed from trees later in the season, catkins were used (Cheng et al., 1997). For 16 progenies from crosses made in 2000, young leaves were collected from the nursery row in Summer 2004, transported to Corvallis, and DNA was extracted as described by Lunde et al. (2000). For DNA samples collected from the 1993 seedlings, PCRs were performed in a volume of 15 μ L containing 10 mM of Tris-HCl (pH 9.0), 50 mM KCl, 0.15% nonionic surfactant (Triton X-100; Sigma Chemical Co., St Louis), 1.5 mM MgCl₂, 120 μ M each of dATP, dCTP, dGTP, and dTTP, 0.1 μ M of primer, 3–25 ng of DNA template, and 0.75 units of Taq polymerase (Promega, Madison, Wis.) (Davis, 1998). Reactions were run simultaneously using a Geneamp PCR System 9700 thermal cycler (Perkin-Elmer Corp., Foster City, Calif.). The parents of each progeny and a control containing no DNA were included. The thermal cycler program consisted of denaturation for 1 min at 94 °C, followed by 5 cycles of 1 min at 94 °C, 1 min 30 s at 37 °C, 30 s at 54 °C and 2 min at 72 °C, and then 35 cycles of 15 s at 94 °C, 45 s at 37 °C, 30 s at 54 °C, and 1 min 30 s at 72 °C. Primer extension occurred for 7 min at 72 °C, and then samples were held at 4 °C until they were retrieved. DNA from each tree was amplified using decamer primer UBC 152 (University of British Columbia, Vancouver, B.C., Canada). Amplification products were separated on 1.5% or 2% agarose gels, stained with ethidium bromide, visualized with a transilluminator, and photographed. RAPD markers are denoted by the primer name followed by subscripts denoting the size of the polymorphic band that each amplifies (UBC152₈₀₀).

For seedlings from 16 progenies from crosses made in 2000, PCRs were performed in a volume of 15 μ L containing the same components and 0.3 μ M of primer, 3–25 ng of template DNA, 0.4 units of Biolase DNA polymerase (Biolase USA, Randolph, Mass.) and the ammonium-based buffer supplied by the manufacturer. Primers UBC 152 and 268 were used.

MARKER CLONING. DNA samples from ‘Zimmerman’, ‘Gasaway’, and OSU 350.089 were used in 15 μ L PCRs with primer UBC 152. PCR samples were compiled and dried down or ethanol precipitated. Ten microliters of the concentrated UBC 152₈₀₀ fragment dissolved in autoclaved nano-filtered water were run on 2% low-melting point agarose (NuSieve; Cambrex Bio Science Rockland, Inc., Rockland, Maine) gels. The fragments were excised from the gels with a sterile blade and purified with the QIAquick kit (Qiagen, Inc., Valencia, Calif.) according to the manufacturer’s instructions. This purified DNA fragment was poly-A tailed to facilitate ligation. Poly-A tailing was performed by combining 30 mM Tris-HCl (pH 7.3), 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, 0.2 mM dATP, 5 units of Taq polymerase, 6 μ L of purified fragment (about 70 ng), and 2.5 mM MgCl₂ in a volume of 10 μ L and

incubating for 30 min at 70 °C. Ligation reactions contained 30 mM Tris-HCl (pH 7.3), 10 mM MgCl₂, 10 mM DTT, 1 mM ATP, 3 units ligase, about 40 ng purified insert, and 50 ng pGEM-T Easy Vector System (Promega). The reactions were stored overnight at 4 °C. JM109 competent *lacI*gZAM15 *Escherichia coli* cells were transformed with the vector which contained an ampicillin resistance gene and the *lac* operon as selectable markers. Putative transformants were grown on LB + ampicillin media with X-Gal and IPTG for blue/white screening. The presence of the desired insert was confirmed with PCR and by digestion with *EcoRI* (0.5 U of enzyme per 50 ng DNA at 37 °C for 1 h) to cut the plasmid at sites flanking the position where the insert should be. After electrophoresis, the fragment size was estimated by comparison with a 100-bp ladder (Promega). Only colonies with an insert of the correct size were multiplied by overnight incubation in LB broth. Plasmid DNA was then isolated from these cultures with the QIAprep Spin Plasmid Miniprep kit (Qiagen) as described by the supplier. The concentration of the purified plasmid DNA was measured with a Hoefer DyNAQuant 200 fluorometer (Hoefer Pharmacia Biotech Inc., San Francisco) and sequenced on an ABI 373A automated sequencer (Applied Biosystems, Perkin-Elmer, Foster City, Calif.). Sequences were edited using GDE (Smith et al., 1994). Alignment, sequence homology, and restriction enzyme sites were analyzed using GCG version 9 (University of Wisconsin Genetics Computer Group, Madison).

POLLEN GERMINATION TEST. Elongated catkins were collected from ‘Barcelona’, ‘Zimmerman’, and ‘Gasaway’ in late Jan. 1999 and laid out on paper overnight. The anthers dehisced and the pollen was poured into glass vials, plugged with cotton, and frozen until the germination test was performed. Germination medium was prepared as described by Kim et al. (1985). Pollen was sprinkled over petri dishes with quadrant dividers. Three plates were made for each genotype and plates were randomized on the bench top and left overnight (12–15 h) at about 21 °C. Pollen grains were scored as having germinated if pollen tubes were longer than the diameter of the grain. One reading of approximately 100 grains was made per quadrant and recorded as percent germination.

Results

Resistant phenotypes represented about 83% of the total in the three progenies from crosses made in 1992. The observed segregation fit a ratio of 3 resistant:1 susceptible in all three progenies (Table 1), but the pooled data showed a slight surplus of resistant seedlings. The percentages of resistant seedlings are greater than the 1:1 that would be expected if ‘Zimmerman’ were heterozygous at a single locus. A total of nine trees, including at least one in each progeny, displayed an intermediate phenotype (Table 1). On these trees, the cankers were small and nonsporulating, and hyphae could not be detected with the ELISA technique or with microscopic examination of stained tissue. The UBC 152₈₀₀ marker was present in all nine plants. These seedlings were considered resistant in goodness-of-fit tests. When seedlings with intermediate phenotypes were excluded, the fit to a 3:1 ratio was better but the ratio was still far from 1:1.

Categorization by phenotype was congruent using the ELISA technique and scoring in the nursery, indicating that the lower-than-expected frequency of infected plants was not due to a large number of disease escapes or error in technique. Furthermore, a second test was performed on all seedlings that were scored as resistant in the first test. The RAPD marker UBC 152₈₀₀, which is

Table 1. Response to greenhouse inoculation of three hazelnut progenies with *Anisogramma anomala* spores and goodness of fit to 3:1 ratio.

Progeny no.	Parents	Disease response (no. trees)			χ^2	P
		Resistant	Small cankers ²	Susceptible		
92004	OSU 55.129 x 'Zimmerman'	42	1	7	3.23	0.072
92005	OSU 342.019 x 'Zimmerman'	34	5	10	0.55	0.458
92006	OSU 350.089 x 'Zimmerman'	39	3	8	2.16	0.142
Pooled data		115	9	25	5.37	0.020

²Trees with small cankers but no sporulating stromata were considered resistant.

Table 2. Co-segregation of disease response and presence of RAPD marker UBC 152₈₀₀ in three hazelnut progenies inoculated with *Anisogramma anomala* spores in the greenhouse.

Progeny no.	Parents	Disease response (no. trees)				Fraction recombination
		Resistant		Susceptible		
		Present	Absent	Present	Absent	
92004	OSU 55.129 x ‘Zimmerman’	43	0	1	6	0.02
92005	OSU 342.019 x ‘Zimmerman’	39	0	2	8	0.04
92006	OSU 350.089 x ‘Zimmerman’	42	0	3	5	0.06
Pooled data		124	0	6	19	0.04

linked to the dominant resistance allele in 'Gasaway', segregates in almost exactly the same proportion as the resistant phenotype, exhibiting only 2%, 4%, and 6% recombination between the marker and the resistance phenotype in the three populations, respectively (Table 2). The DNA sequences of the UBC 152₈₀₀ RAPD markers in 'Zimmerman' and 'Gasaway' were nearly identical (99.25% identity, data not shown). UBC 152₈₀₀ from 'Gasaway' is GenBank accession no. CC875208.

Some postzygotic mechanisms were investigated but discounted as explanations for the low incidence of infection. Nut germination and transplanting records did not provide evidence of selection in favor of resistant seedlings. Two progenies (92005 and 92006) included nondormant seedlings (Thompson et al., 1985), most of which were rogued prior to planting in the field. The remainder of the nondormant seedlings suffered cold injury in the field. The third progeny, 92004, does not segregate for nondormancy, but gave a proportion of resistant plants similar to that in the other two (Tables 1–2). Thus it does not appear that the high frequency of resistant seedlings was due to selection against nondormants. Likewise, the records of moldy or rotted kernels, stunted seedlings, seedlings with roots but no shoots, or twins failed to indicate that selection during stratification or transplanting was responsible for the high percentage of resistant seedlings. The stratification procedure is fairly long, yet the similarities in the percentage of resistant seedlings make it unlikely that one of these was the cause of the observed surplus of resistant seedlings.

Pollen germination was 29% for 'Zimmerman', 71% for 'Gasaway', and 18% for 'Barcelona', indicating that selection at the gametophytic level was possible.

Sixteen resistant seedlings of 'Zimmerman' were crossed with the highly susceptible selection OSU 313.078. Segregation fit a 3:1 ratio in 14 of the 16 progenies, and showed a surplus of resistant seedlings in the other two (Tables 3–4). No progeny showed a 1:1 segregation ratio. OSU 313.078 produces no nondormant offspring. Resistance co-segregated with the two RAPD markers (UBC152₈₀₀ and UBC268₅₈₀) that flank the 'Gasaway' resistance allele (Table 3). Of 18 selections from a cross of VR 6-28 x 'Zimmerman', only one was susceptible. This fits a 7:1 ratio although the sample size is small.

To test allelism of resistance from 'Gasaway' and 'Zimmerman', eight selections from a cross of VR 6-28 x 'Zimmerman' were

crossed with OSU 313.078. Five of the eight progenies segregated 3:1, two progenies segregated 1:1, and OSU 313.078 x OSU 720.056 gave only resistant offspring (Table 5). This indicates that OSU 720.056 is homozygous resistant and that 'Zimmerman' and 'Gasaway' share a common resistance allele.

Discussion

After observing a high frequency of resistant seedlings in three progenies, we looked at segregation distortion as an explanation. The phenomenon is common, affects diverse types of plants including annuals and perennials, and it is likely that there are many mechanisms behind it. It has been reported in perennial crops including poplar (*Populus* L.) (Bradshaw and Stettler, 1994), douglas fir (Jermstad et al., 1994), eucalyptus (*Eucalyptus* L'Hér.) (Grattapaglia and Sederoff, 1994), cherimoya (*Annona cherimola* Mill.) (Perfectti and Pascual, 1996), and avocado (*Persea americana* Mill.) (Torres et al., 1986). In hazelnut, distortion was found to be common in isozyme analyses (Cheng, 1992; Rovira et al., 1993). Clonally propagated crops seem especially prone to distortion, and within this group distortion has been attributed to genetic load (Bradshaw and Stettler, 1994) or accumulation of somatic mutations (Klekowski, 1988).

There are two possible explanations for the unexpectedly high frequency of resistant genotypes in these populations. One is that 'Zimmerman' indeed inherited resistance from 'Gasaway', but that some mechanism of segregation distortion is acting in favor of resistant genotypes in seedling populations. Selection pressure on male or female germline cells or postzygotic selection pressure before the trait of interest is evaluated will affect segregation. These can be genes important during sporogenesis, for proper functioning of germ cells, for seed development, for germination, or for plant growth (Xu et al., 1997; Zamir and Tadmor, 1986). Similarly, artificial selection for traits linked to these pre- or postzygotic factors will distort segregation.

One cause of prezygotic selection is meiotic drive. This is the preferential retention of one member of a pair of heterozygous alleles or one heteromorphic chromosome such that it is transmitted to more than half of the resulting meiocytes (Lyttle, 1991). Chromosome loss can also function during meiosis but is most common in interspecific crosses (Bradshaw and Stettler, 1994).

Table 3. Segregation for eastern filbert blight disease scores and presence of two RAPD markers in 16 hazelnut progenies. Potted trees were exposed to eastern filbert blight under structures topped with diseased wood. Highly susceptible selection OSU 313.078 was the female parent of all progenies. All pollen parents are seedlings of 'Zimmerman'.

Progeny no.	Male parents	Disease response	Resistant	Small cankers	Resistant	Resistant	Susceptible	Susceptible	Susceptible
		UBC 152-800	Present	Present	Absent	Present	Absent	Absent	Present
		UBC 268-580	Present	Present	Present	Absent	Absent	Present	Absent
	Male from OSU 350.089 x ‘Zimmerman’								
00031	653.016	62	5	1	0	10	2	0	
00037	669.066	57	3	2	0	16	2	0	
00038	669.070	57	0	0	1	20	2	0	
00039	Epsilon	17	1	1	0	4	0	0	
	Male from OSU 342.019 x ‘Zimmerman’								
00032	659.010	32	4	1	1	18	3	0	
00033	659.022	49	3	4	0	9	3	0	
00034	659.033	48	4	0	0	19	1	1	
00035	659.044	67	1	1	0	7	1	0	
00036	659.046	53	1	0	0	23	1	1	
00040	669.094	58	3	0	1	17	1	0	
00041	669.095	49	7	2	0	20	2	0	
00042	669.104	52	2	2	0	21	1	1	
00043	669.113	52	5	0	2	19	0	0	
00044	Zeta	46	1	2	0	9	0	0	
00045	670.098	36	1	1	0	15	1	0	
00046	670.106	51	3	3	1	19	2	0	

Table 4. Goodness-of-fit tests to a 3:1 ratio for disease response and presence of two RAPD markers in 16 hazelnut progenies. Potted trees were exposed to eastern filbert blight under structures topped with diseased wood. All pollen parents are seedlings of 'Zimmerman'.

Progeny	Disease response ^z					UBC 152-800				UBC 268-580			
no.	Parents	Resistant	Susceptible	χ^2	<i>P</i>	Present	Absent	χ^2	<i>P</i>	Present	Absent	χ^2	<i>P</i>
	Male from OSU 350.089 x 'Zimmerman'												
00031	313.078 x 653.016	68	12	4.27	0.04	67	13	3.27	0.07	70	10	6.67	0.01
00037	313.078 x 669.066	62	18	0.27	0.61	60	20	0.00	1.00	64	16	1.07	0.30
00038	313.078 x 669.070	58	22	0.27	0.61	58	22	0.27	0.61	59	21	0.07	0.80
00039	313.078 x Epsilon	19	4	0.71	0.40	18	5	0.13	0.72	19	4	0.71	0.40
	Male from OSU 342.019 x 'Zimmerman'												
00032	313.078 x 659.010	38	21	3.53	0.06	37	22	4.75	0.03	40	19	1.63	0.20
00033	313.078 x 659.022	56	12	1.96	0.16	52	16	0.08	0.78	59	9	5.02	0.03
00034	313.078 x 659.033	52	21	0.55	0.46	53	20	0.22	0.64	53	20	0.22	0.64
00035	313.078 x 659.044	69	8	8.77	0.00	68	9	7.28	0.01	70	7	10.39	0.00
00036	313.078 x 659.046	54	25	1.86	0.17	55	24	1.22	0.27	55	24	1.22	0.27
00040	313.078 x 669.094	62	18	0.27	0.61	62	18	0.27	0.61	62	18	0.27	0.61
00041	313.078 x 669.095	58	22	0.27	0.61	56	24	1.07	0.30	60	20	0.00	1.00
00042	313.078 x 669.104	56	23	0.71	0.40	55	24	1.22	0.27	57	22	0.34	0.56
00043	313.078 x 669.113	59	19	0.02	0.90	59	19	0.02	0.90	57	21	0.15	0.69
00044	313.078 x Zeta	49	9	2.78	0.10	47	11	1.13	0.29	49	9	2.78	0.10
00045	313.078 x 670.098	38	16	0.62	0.43	37	17	1.21	0.27	39	15	0.22	0.64
00046	313.078 x 670.106	58	21	0.11	0.75	55	24	1.22	0.27	59	20	0.00	0.95

^aTwo seedlings in progeny 00035 and one seedling in progeny 00042 showed no disease but lacked the two flanking markers. These were considered escapes and not included in this table.

Maternal abortion or competition between megaspores can cause distorted segregation. However, the first three progenies were produced by unrelated seed parents, decreasing the likelihood that this mechanism functions in the 'Zimmerman' progenies.

Another cause of prezygotic selection is certation, or abnormal pollen function. Competition due to differential pollen vigor acts from pollen germination through fertilization. In *Lycopersicon hirsutum* Humb. and Bonpl., genes expressed in pollen give advantage in low temperatures, exemplifying that even the haploid

genome is subject to selection (Zamir et al., 1982). In European hazelnut, fertilization takes place 4–5 months after pollination. The pollen tube grows toward the ovule and forms a callosed resting stage; fertilization eventually occurs once the ovule has formed (Thompson, 1979). It is conceivable that pollen genotypes having lesser ability to complete this process would be selected against. If 'Zimmerman' is heterozygous at a single resistance locus and the pollen carrying the susceptible allele is linked to allele(s) causing poor pollen fitness, susceptible genotypes would

Table 5. Segregation for disease response in eight hazelnut progenies to test allelism of 'Gasaway' and 'Zimmerman' resistance. Potted trees were exposed to eastern filbert blight under structures topped with diseased wood. Highly susceptible selection OSU 313.078 was the female parent of all progenies.

Progeny no.	Male parent ^z	Disease response (no. trees)			Resistant (%)	χ^2 (1:1)	<i>P</i> (1:1)	χ^2 (3:1)	<i>P</i> (3:1)	Best fit
		Resistant	Small cankers ^y	Susceptible						
00048	703.062	60	0	20	75.0	20.00	0.000	0.00	1.000	3:1
00049	703.068	54	3	22	72.2	15.51	0.000	0.34	0.559	3:1
00050	703.076	62	2	15	81.0	30.39	0.000	1.52	0.217	3:1
00051	703.104	39	1	39	50.6	0.01	0.910	25.02	0.000	1:1
00052	703.109	36	0	10	78.3	14.70	0.000	0.26	0.610	3:1
00053	720.056	78	2	0	100.0	80.00	0.000	26.67	0.000	1:0
00054	720.057	37	1	14	73.1	11.08	0.001	0.10	0.749	3:1
00055	720.059	48	2	31	61.7	4.46	0.035	7.61	0.006	1:1

^zThe pollen parents are resistant seedlings from a cross of VR 6-28 x 'Zimmerman'. VR 6-28 is from a cross of 'Riccica di Talanico' x 'Gasaway' and carries the dominant allele for eastern filbert blight resistance from 'Gasaway'.

^ySeedlings with small cankers and nonsporulating stromata were classified as resistant for goodness-of-fit tests.

be compromised in the offspring resulting from its use as a pollen parent. The low rate of germination of 'Zimmerman' pollen may indicate that this is the cause of distortion in these populations. Pollination germination in 'Zimmerman' was low and similar to the 40% reported for 'Barcelona' by Saleses (1973). This mechanism has been cited as a possible cause of distortion in the inheritance of isozymes in hazelnut (Rovira et al., 1993).

Some postzygotic mechanisms were discounted. No trend could be seen in the records of moldy or rotted kernels, stunted seedlings, seedlings with roots but no shoots, or twins indicating that selection during stratification or transplanting is not likely responsible for the high percentage of resistant phenotypes in these three progenies. The stratification procedure is fairly long, yet the similarities in the percentage of resistant genotypes make it unlikely that one of these is the cause of the observed distortion. Nondormant mutants were present in two of the 1992 'Zimmerman' populations, and although progeny 92004 does not segregate for nondormancy, the proportion of resistant plants in all three populations is similar (Tables 2–3). None of the progenies produced in 2000 segregated for nondormancy, yet nearly all gave a high frequency of resistant seedlings.

The likelihood of misclassification of disease phenotypes is slight because field data and greenhouse data for the 1992 progenies were consistent (Table 3). Inoculations of resistant seedlings were repeated and confirmed. A similar percentage (83%) of resistant genotypes was previously reported in other plants from population 92004 (Coyne, 1995). Furthermore, the disease and RAPD marker phenotypes were in excellent agreement. The UBC 152₈₀₀ marker is well-characterized and its co-segregation with the resistance phenotype in the three progenies is not due to improper scoring. Furthermore, the same 3:1 ratio was observed in the testcross populations. The UBC 268₅₈₀ marker is similarly robust and co-segregated with resistance in the 16 progenies.

Chromosome translocation has been cited as a cause of low pollen viability in certain cultivars. Saleses (1973) observed quadrivalents and trivalents in pollen mother cells of 'Barcelona', 'Tonda Gentile delle Langhe', and 'Segorbe' in meiosis I. Further study revealed that the chromosome pair involved in the reciprocal translocation in 'Barcelona' was the same in 'Segorbe', 'Negret', and 'Tonda Gentile delle Langhe', but that a different translocation was present in 'Tonda di Giffoni' (Saleses and Bonnet, 1988). Because of cytogenetic abnormalities, 'Zimmerman' may have inherited two copies of the chromosome region that contains the resistance locus and flanking RAPD markers from 'Gasaway'.

If resistance were linked to two independent centromeres, a 3:1 segregation ratio for disease response and flanking markers would be expected. Recently, Gökirmak (2005) investigated 270 hazelnut accessions using 21 simple sequence repeat markers. At all 21 marker loci, 'Zimmerman' had one allele in common with 'Barcelona' and one allele in common with 'Gasaway'. This is strong evidence that 'Barcelona' and 'Gasaway' are the parents of 'Zimmerman'.

Our hypothesis is that 'Zimmerman' is heterozygous ($R_1 r_1 R_2 r_2$) at two independent loci and that only plants homozygous recessive at both loci are susceptible. This would explain the observed 3 resistant:1 susceptible ratio in the 1993 progenies. Following this reasoning, crosses of resistant 'Zimmerman' progeny from the 1993 crosses with susceptible plants should segregate 3 resistant:1 susceptible or 1 resistant:1 susceptible. However, all 16 crosses of 'Zimmerman' seedlings to OSU 313.078 showed an excess of resistant seedlings, and 14 fit a 3:1 ratio (or higher). None exhibited a 1:1 ratio. In all 16 progenies, segregation for two flanking RAPD markers was highly correlated with disease response (Table 3).

Following the two-locus model, seedlings from a cross between VR 6-28 which carries the 'Gasaway' resistance allele and 'Zimmerman' should segregate 7 resistant:1 susceptible. The observed 17 resistant:1 susceptible ratio fits the expected ratio. When selections from this progeny were crossed with OSU 313.078, three segregation ratios were seen in the offspring: 3:1, 1:1 and 1:0. If the UBC 152₈₀₀ marker were linked only to the resistance allele from 'Gasaway', the marker should segregate 1 present:1 absent, but 3:1 ratios were observed. In order to explain these results, we hypothesize that the second locus arose from duplication of the first and that the flanking regions that produce the UBC152₈₀₀ and UBC268₅₈₀ markers were also duplicated. It is interesting to note that in the 1992 crosses, the only recombinants seen were susceptible plants with the marker. In the 2002 crosses, however, four types of recombinants were found (Table 3).

Resistance from 'Gasaway' and 'Zimmerman' has been called "immunity" or "complete resistance." However, we noted a few seedlings with small cankers but nearly all lack sporulating stromata. A few seedlings produce occasional stromata. Flanking RAPD markers were present, indicating that the resistance allele is also present in these seedlings. So although not "immune" or "completely resistant," 'Gasaway' and 'Zimmerman' transmit a very high level of resistance.

In summation, it is hoped that increasing knowledge about novel sources of resistance to EFB will aid in the development of highly resistant cultivars. Investigation of EFB resistance in progenies of 'Zimmerman' has provided us with very useful information. Because it appears that 'Zimmerman' carries the same resistance allele as 'Gasaway', albeit two independent copies, it does not provide us with a new resistance gene, but we now know that we can use our RAPD markers in progenies segregating for resistance from 'Zimmerman'. In addition, the proportion of resistant progeny in crosses of 'Zimmerman' is much higher than when 'Gasaway' is used as the resistance source. Although 'Zimmerman' has many undesirable traits, it is a significant improvement over 'Gasaway' with respect to nut yield, nut size, and nut shape and is thus useful as a parent in breeding for disease resistance.

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