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 Weschke selections TP1, TP2 and TP3; C. colurna × C. avellana hybrids Chinese Trazela Gellaty #6 and #11; Turkish Trazel Gellaty #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.D.C.) 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 pollinator ‘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–9.9K) (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 stroma 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 stroma 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
genotypes, most of which were numbered & scored as negative by ELISA and no symp-
as completely resistant if all three trees were
was scored as positive by ELISA or if symp-
as susceptible if one or more of the three trees
sayed 6 months later. An accession was scored
was as susceptible if one or more of the three trees
scored as positive by ELISA or if symp-
as negative by ELISA and no symp-
tons were measured
arried out, and were reas-
was scored as positive by ELISA or if symp-
as negative by ELISA and no symp-
tons were measured
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 Mehlen-
bacher 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 pro-
vided 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 resis-
tant), Lewis (moderately resistant), and
Tonda di Giffoni (high quantitative resis-
tance). Trees of selection OSU 729.012,
‘Ratoli’, ‘Closca Molla’, and ‘Santiam’; and four
recently released pollinator cultivars
(Gamma, Delta, Epsilon, and Zeta) were
included. 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 × (C. californica × 729.012) × OSU
14.084 (‘Barcelona’ × ‘Daviana’) and OSU
350.089 (‘Tombul Ghiagli’ × ‘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
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
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
developed cankers of various lengths
after 16 to 18 months (Table 3). The negative
control ‘Gasaway’ and the positive controls
‘Emm’ 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, ‘Culpa’
and CCOR 187, remained free of infection.
‘Culpa’ 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 resistent to B. tabaci
(Pythytos apterus var. 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).

| Cultivar                  | No. of trees | Exposed | Infected | Canker length | No. of cankers |
|--------------------------|-------------|--------|---------|---------------|---------------|
| Daviana                  | 12          | 12     | 13      | 13.05         | 7             |
| 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      | 4.5     | 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           |               |
| LSD0.05                  | 2.03        |        |         | 1.8           |               |

*aNumber of potted trees exposed and number infected with eastern filbert blight. For most genotypes in the
test, 12 potted trees were exposed.
*bMean of total canker length per tree, expressed on a square root scale. Cankers were measured on 5, 6, and
10 Jan. 2006.
*cMean 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.

Table 2. Hazelnut accessions resistant to<br>Antisogramma anomala after greenhouse<br>inoculation and their origins.

| Accession | CCOR | Origin       |
|-----------|------|--------------|
| C. avellana |     |              |
| Culpa     | 255  | Spain        |
| CCOR 187  | 187  | Finland      |
| C. americana hybrids | |              |
| CCOR 507  | 507  | Minn., US    |
| G081S     | —    | Minn., US    |
| Weschcke TP1 |    | Wisc., US    |
| Weschcke TP2 |    | Wisc., US    |
| Weschcke TP3 | 561 | Wisc., US    |
| C. colurna hybrids | |              |
| Chinese Trazel | |              |
| Gellatly #6 | 138 | B.C., Canada |
| Gellatly #1 | 173 | B.C., Canada |
| Gellatly #3 | 407 | B.C., Canada |
| Lisa      | —    | Mich., US    |
| C. heterophylla var. sutchuenis | |              |
| × C. avellana ‘Holder’ | |              |
| Estrella #1 | 59  | Mich., US    |

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

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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. *avellana* 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 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. *sutchuenensis* with C. *avellana* ‘Holder’ (Farris, 1982). Estrella #1 showed complete resistance. 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 pus-tules. 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 *C. colurna* 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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