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Sources of resistance to eastern filbert blight in hazelnuts from the Republic of Georgia

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ABSTRACT

In North America, the native hazelnut *Corylus americana* harbors an endemic fungus, *Anisogramma anomala*, which causes the disease eastern filbert blight (EFB). Although *C. americana* is tolerant of EFB, the economically important European hazelnut, *C. avellana*, is highly susceptible to the disease. This susceptibility greatly limits commercial European hazelnut production in North America. The breeding and subsequent utilization of resistant plants are considered the most effective means of control and efforts are underway to identify diverse sources of genetic resistance. In this study, 1374 *C. avellana* seedlings, spanning 47 seed lots collected in the Republic of Georgia and 3 in Azerbaijan, were inoculated with the EFB pathogen and evaluated for disease response in New Jersey, USA. After 5 years, plant responses were rated on a scale of 0–5, in which 0 represents no signs or symptoms of EFB and 5 represents all stems showing cankers. Cankers were found on over 94% of the seedlings, with the large majority being highly susceptible (91.4% = rating 4 and 5). However, 79 plants from 34 seed lots remained free of signs or symptoms of EFB (rating 0). In most cases, only one or two resistant seedlings were in each of these seed lots, making it difficult to infer any genetic control of resistance. In contrast, seed lots from 'Gulshishvela' and 'Kharistvala' yielded significant numbers of resistant plants, which may indicate that dominant genes for resistance are present. Overall, these new plants will add to the genetic resources available for breeding new EFB-resistant cultivars.

1. Introduction

Eastern filbert blight (EFB) is an endemic disease of hazelnuts (*Corylus* spp.) in North America caused by the ascomycete fungus *Anisogramma anomala*. The fungus persists in association with the wild American hazelnut (*Corylus americana*), the range of which reaches across much of the United States and southern Canada, east of the Rocky Mountains (Gleason and Cronquist, 1998). Despite its typical insignificance to the health of *C. americana* (Capik and Molnar, 2012; Fuller, 1908; Weschcke, 1954), in the commercially valuable European relative, *C. avellana*, it induces severe cankering, stem dieback, and subsequent death of most cultivars (Johnson and Pinkerton, 2002). Consequently, this disease is the primary factor that restricts commercial hazelnut production in the eastern United States (Thompson et al., 1996).

Eastern filbert blight was not present in the US Pacific Northwest when the hazelnut industry developed there at the turn of the 20th century (Barss, 1930). Today, 99% of the current US hazelnut crop is produced in the Willamette Valley of Oregon, representing just 3.44% of worldwide production, which was estimated at 915,846.40 t in 2012 (Food and Agricultural Organization of the United Nations, 2012). The Mediterranean-like climate of the region supported successful *C. avellana* production for nearly a century, until the 1960s, when *A. anomala* was introduced into western Washington state (Davison and Davidson, 1973). The spread of EFB into the region caused dramatic changes to production there, where orchards were devastated due to a lack of effective control measures (Gottwald and Cameron, 1980; Pinkerton et al., 1992). The disease can now be found throughout much of the Willamette Valley and is effectively present in all regions where hazelnuts can be grown in North America.

Susceptible orchards can be managed through scouting for cankers, remedial pruning, and profuse applications of fungicide (Johnson et al., 1996). This arduous management regimen is both expensive and time-consuming, undermining the traditional low-input nature of hazelnuts. As such, the development of EFB-

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resistant cultivars is a much more cost-effective and sustainable solution (Julian et al., 2008, 2009; Thompson et al., 1996). Accordingly, identifying sources of genetic resistance and using them to develop improved cultivars has become a primary focus of hazelnut breeding programs in the US (Mehlenbacher, 1994; Molnar et al., 2005). Of the recognized *Corylus* species, *C. avellana* produces the largest nuts with the most desirable kernels, compared to other species that are generally small, thick-shelled, and of little economic interest (Mehlenbacher, 1991). The identification of plants resistant to EFB with commercial nut quality would expedite the breeding of superior cultivars for use where the disease is present. Pursuing EFB resistance within *C. avellana* is therefore the most favorable path toward developing commercially viable cultivars for production in the US.

In the 1970s, *C. avellana* 'Gasaway' – a late-shedding pollinizer with poor nut qualities and low yields – was the first European hazelnut found to be resistant to EFB. It was subsequently shown to transmit a dominant allele at a single locus that confers resistance to its offspring in a ratio of 1 resistant:1 susceptible (Mehlenbacher et al., 1991a). After decades of modified backcross breeding at Oregon State University (OSU), Corvallis, OR, the 'Gasaway' *R*-gene was successfully introgressed into plants of commercial quality resulting in the release 'Yamhill' (Mehlenbacher et al., 2009), 'Jefferson' (Mehlenbacher et al., 2011), 'Dorris' (Mehlenbacher et al., 2013), and 'Wepster' (Mehlenbacher et al., 2014). Based primarily on cultivars carrying the 'Gasaway' *R*-gene (mostly 'Jefferson'), the Oregon industry has expanded by ~6000 ha over the past 5 years (Mehlenbacher, personal communication, November 19, 2014).

Despite the widespread use of 'Gasaway', concern about the long-term durability of the single *R*-gene led researchers at OSU to seek out additional resistant plants. Various *C. avellana* cultivars, seedlings, and interspecific hybrids have since displayed resistance to EFB in Oregon and are now being used in breeding. The most notable EFB-resistant *C. avellana* include 'Ratoli' and 'Culpla' from Spain, 'Crevenje' and 'Uebov' from Serbia, OSU 495.027 from southern Russia, OSU 408.040 from Minnesota, USA, CCOR 187 from Finland, and OSU 759.010 from Georgia, which is discussed in more detail later in this article (Chen et al., 2005, 2007; Colburn et al., 2015; Coyne et al., 1998; Lunde et al., 2000; Sathuvalli et al., 2009, 2010, 2011a,b). Additional EFB-resistant seedling selections from Turkey, Russia, and Crimea have also more recently been identified at OSU that merit further testing (Mehlenbacher, personal communication, November 19, 2014). Further, collection and evaluation efforts at Rutgers University have identified EFB-resistant seedlings from Russia, Crimea, Poland, Moldova, Turkey, Estonia, Latvia, and Lithuania, as well as various grower selections and other interspecific hybrids (Capik and Molnar, 2012; Capik et al., 2013; Molnar et al., 2007; Molnar, unpublished). Thus, EFB-resistant hazelnuts span a number of geographic origins, and in studies using simple sequence repeat (SSR) markers, Gökirmak et al. (2009) and Muehlbauer et al. (2014a) showed that many of them are genetically diverse and unrelated.

While this progress is promising, recent studies at Rutgers University have shown that *A. anomala* is genetically diverse (Cai et al., 2013; Muehlbauer et al., 2014b, unpublished) and may also exhibit pathogenic variation; various hazelnut genotypes known to be resistant in Oregon developed EFB when challenged with eastern USA isolates (Capik and Molnar, 2012; Molnar et al., 2010a,b). Although the studies also showed that a number of different hazelnut genotypes remained apparently unaffected when exposed to a diversity of isolates, these results underscore the importance of seeking additional sources of genetic resistance for use in breeding.

Hazelnuts have been cultivated in Turkey and the Mediterranean region since classical times (as early as 500 bc) and at a much earlier time in the Black Sea region of Turkey and the Caucasus (Thompson et al., 1996). The Balkan Peninsula has also been cited as an important center of diversity for *C. avellana* (Bošnjaković et al., 2012). Hazelnuts are an ancient crop in the Republic of Georgia, where commercial and backyard garden production is still common and the forests also hold wild trees, making it a promising location to be explored for hazelnut genetic resources. The fifth largest producer in the world, 24,700 t of hazelnuts was produced in the Republic of Georgia in 2012 (Food and Agricultural Organization of the United Nations, 2012), with extensive new plantings occurring in recent years (Pisetta, personal communication, July 5, 2015).

In this study, a wide range of hazelnut germplasm was collected across the Republic of Georgia and the resulting seedlings planted in the field in New Jersey, USA. The objective was to subject these seedlings to high EFB pressure, in order to evaluate their response to the disease and identify potential new sources of genetic resistance.

2. Materials and methods

2.1. Plant material

Hazelnut germplasm (in the form of open-pollinated, fresh, in-shell nuts) was collected by Michele Pisetta from locations across the Republic of Georgia in August 2009, selecting for good nut quality (round kernels, a kernel-to-shell ratio close to or over 50%, and freedom from defects) when possible. Sixty-two seed lots were obtained or purchased – some of which represent multiple but distinct accessions from one location – from roadside vendors, markets, private growers, experimental gardens, and the wild (Molnar and Pisetta, 2009). Three of the seed lots were collected across the border in Azerbaijan (Balakan and Zaqatala). Hazelnuts are self-incompatible and wind pollinated (Mehlenbacher, 1991). Thus, open-pollinated seed presents an opportunity to capture a wide diversity of recombinant genotypes from a region including wild trees not located directly in the orchard. Their origins and/or descriptions, as well as seed parent cultivar name when known, are listed in Table 1. The identification numbers in Table 1 correspond to the collection locations (Fig. 1).

The nuts were cleaned and air dried in Georgia, prior to phytosanitary inspection and shipping. Upon receipt, nuts were stored in polyethylene bags in cold storage (4 °C) until October 2009. They were then provided a moist chilling period in polyethylene bags of damp peat at 4 °C for 5 months. In March 2010, they were germinated in wooden planting boxes filled with peat-based growing medium (Promix® BX; Premier Horticulture, Rivière-du-Loup, Québec, Canada) in a greenhouse maintained at 24 °C/18 °C day/night with 16-hour daylengths. After 4–6 weeks, seedlings were transplanted to individual 3.7-L plastic pots with the same growing medium and top-dressed with 5 g of 5–6 month time-release fertilizer (Osmocote Plus 15N-9P_2O_5-12K_2O with micronutrients; The Scotts Co., Marysville, OH).

2.2. Greenhouse inoculations

At 8–10 weeks after germination, the seedlings were placed into polyethylene covered chambers kept at ~100% relative humidity and inoculated with an *A. anomala* ascospore suspension, prepared and applied as described in Molnar et al. (2007). Once the inoculated seedlings were removed from the chambers, they were maintained under optimal growing conditions in the greenhouse. In late June 2010, they were moved outside for acclimation under 40% shade, until field planting in October 2010 at the Rutgers University Horticultural Farm 1 in New Brunswick, NJ. Trees were spaced 0.5 m apart in-row and 3.0 m between rows. Seedlings were planted in groups by progeny code. Weed control using herbicides, irrigation, and annual applications of fertilizer were all provided as needed, with no use of pesticides or fungicides.