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Results and Implications Following a Twelve-Year Monitoring of Ash (*Fraxinus* spp.) Mortality Due to *Agrilus planipennis* in an Urban Forest Plantation

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Abstract. Identifying ash genotypes that are resistant to emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB) continues to be an effort of importance to widen the existing knowledge on interspecific variation in EAB-host preference, identify mechanisms involved in EAB infestation, and aid in development of EAB-resistant ash hybrids that could potentially serve as viable replacements in urban plantations. A research plantation composed of 17 cultivars and 2 seedlings of ash trees was established in Toledo, Ohio, U.S., to monitor the susceptibility of these trees to EAB. The tree mortality was recorded on an annual basis since 2005. The results from the study and its implications, at the end of 12 years of monitoring, are presented in this paper. The study reaffirmed what is known about the resistance of the Asian ash species *Fraxinus mandshurica* ('Mancana') and the general susceptibility of North American ashes to EAB. However, it was observed that the degree of susceptibility to EAB varied across the different cultivars, with white ash cultivars 'Autumn Purple' and 'Rosehill' showing higher survival rates. However, this conclusion is based on a low replication, and all but one individual of each cultivar were infested at the end of the study. The findings suggest a need for continued and thorough screening of ash cultivars for identifying resistant genotypes.

Key Words. *Agrilus planipennis*; Emerald Ash Borer; Exotic Pests; Interspecific Variation; Resistance; Urban Forestry.

Since its discovery in 2002, the emerald ash borer (EAB) has killed many millions of ash trees (*Fraxinus* spp.) in natural and urban forests (Herms and McCullough 2014). The potential multi-billion dollar ($USD) economic impact of an EAB infestation is more pronounced in urban forests of the United States (Macquarrie and Scharbach 2015). In Asia, EAB preferentially attacks stressed trees, including Manchurian ash (*F. mandshurica*) that is native to Asia (Liu et al. 2007). In the United States, even healthy trees in high-quality sites are being attacked (Poland and McCullough 2006). All North American ash species are susceptible to EAB to varying degrees, with ash mortality exceeding 99% in areas surrounding the epicenter of the original EAB infestation in Michigan, U.S. (Klooster et al. 2014). However, a small number of ash trees that were not preferred by EAB, or at least appear to have tolerated EAB infestation better than the rest, still remain. These trees, termed "lingering ash," are of particular interest to researchers involved in identifying mechanisms of ash tree resistance to EAB, as well as to those involved in ash hybridization efforts (Knight et al. 2013; Koch et al. 2015; Villari et al. 2016). Researchers are actively involved in advancing the knowledge of ash tree resistance to EAB and are making efforts to identify research gaps that need to be addressed for better EAB management (Poland et al. 2015; Villari et al. 2016). Research in EAB-host resistance continues to be a critical need, with the hope that it will ultimately produce EAB-resistant cultivars (Herms and McCullough 2014). A cost-effective way to identify or deploy resistant germplasm is evaluating existing genotypes for resistance (Herms 2002). Contrasting findings of the susceptibility of ash species, such as in the case of the commer-

cial *Fraxinus* × 'Northern Treasure' ash, which is a black ash (*F. nigra*) × Manchurian ash hybrid (Herms 2015; Rebek et al. 2008), further underscores the need for continued research attention in this area. In 2004, right when EAB started to impact Toledo, Ohio, U.S., an urban plantation was established there by The Ohio State University Extension; the site was approximately 53 km south of the original EAB infestation (Detroit, Michigan, U.S.). The plantation was established to evaluate the interspecific variation in ash tree mortality due to EAB in several cultivars and seedlings of ash. The trees were monitored for growth and mortality due to EAB on an annual basis. In this short communication, researchers present the results and discuss the research implications stemming from the monitoring effort.

METHODS

The plantation included a total of 156 ash trees, composed of 9 ash species (17 ash cultivars and 2 ash seedlings). Two 30 m wide median strips, approximately 50 m apart from each other, were chosen to plant the trees with a plant spacing of 7.62 m. The plantings were installed using a completely randomized design during April–May in 2004 and 2005, depending on the availability of the planting material. A list of trees planted, their number, and their source is provided in Table 1. Cultivars (named and unnamed; see Table 1) were generally planted as 1.2–1.5 m whips, while seedlings were generally less than 30 cm tall at planting. The trees were never treated with pesticides to control EAB and were never irrigated. Plants were mulched with uncomposted hardwood chips. The trees were evaluated annually (once between June and September) for tree growth [diameter breast height (DBH) measured at 1.3 m off the ground; for trees shorter than 1.3 m, the DBH was noted as zero]. The trees were visually inspected for any apparent symptoms of EAB infestation, including D-shaped exit holes, bark splitting, and serpentine galleries. Each year, the trees were classified as healthy, possibly infested, or dead. A tree was considered dead because of EAB when all the branches or crown above 1.3 m were dead along with the presence of EAB symptoms. Data reported in this paper include years between 2004 and 2016. The data for 2007 was lost during the transition when a student who collected the data graduated from the university. Henceforth, data from 2007 are not presented in the results. Although data on visual symptoms of EAB infestation were collected annually, only data on mortality (i.e., dead versus alive) is presented in this study, as researchers did not conduct an exhaustive survey of EAB infestation.

RESULTS

At the end of the 12-year monitoring period in 2016, the DBH of trees across all ash species ranged between 17 cm and 31 cm, with an average DBH of 24.2 cm. The trees that were planted as true seedlings, including black ash and Manchurian ash, had the smallest average DBH (18.9 cm and 17 cm respectively). The two narrowleaf ash cultivars of European origin (*F. angustifolia* subsp. *angustifolia* cv. 'Desert' and *F. angustifolia* subsp. *oxycarpa* cv. 'Raywood') showed the greatest average diameter growth (30.6 cm and 31 cm, respectively) despite their smaller size at planting (Figure 1). The 'Northern Treasure' ash had an average DBH of 28.7 cm after 12 years. Even though the trees that were planted as seedlings were small at the time of planting, most of the trees had grown to an EAB infestable size of 2.5 cm (Herms and McCullough 2014) by 2008. The trees that were smaller than 2.5 cm in 2008 included one black ash seedling, one blue ash (*F. quadrangulata*) seedling, two green ash seedlings, and three Manchurian ash seedlings. All trees had grown to an infestable size by 2010.

After discounting the 20 trees (13% of the 156 total trees) that were lost due to reasons other than EAB [failure to establish (n = 15), deer and mechanical damage (n = 5)], the overall survival rate in 2016 was 45% (62 trees) (Table 1). In all, 55% of the 136 trees were declared dead from EAB after 12 years. Of the surviving trees, only Manchurian ash and blue ash had all of the planted trees surviving in 2016 (after discounting the three Manchurian ash and two blue ash trees that were lost to reasons other than EAB). Followed by the aforementioned ashes, the 'Northern Treasure' ash, as well as the two white ash (*F. americana*) cultivars 'Rosehill' and 'Autumn Purple', exhibited higher survival rates (83%, 78%, and 71%, respectively; Table 1). Among the black ash cultivars, the 'unnamed cultivar' exhibited higher survival rate (57%) than 'Fall Gold' (0%). Similarly, ash cultivars of European

Table 1. The number of trees planted, the number of trees dead due to EAB, the number of trees still alive but infested with EAB, and the percentage of trees surviving at end of the 12-year monitoring period.

Cultivar	Number at Planting	Number of dead trees due to EAB	Number of trees alive	Number of trees lost to reasons other than EAB	Number of alive trees with apparent EAB symptoms	Percent survival at the end of 12-year monitoring period
Asian (Influence)						
F. mandshurica (seedling)[z]	8	0	5	3	0	100
F. mandshurica cv. 'Mancana'[y]	8	0	8	0	0	100
F. nigra × *mandshurica* 'Northern Treasure'[x]	6	1	5	0	2	83
European						
F. angustifolia subsp. *angustifolia* cv. 'Desert'[x]	7	4	3	0	2	43
F. excelsior cv. 'Golden Desert'[x]	8	2	5	1	3	71
F. angustifolia subsp. *oxycarpa* cv. 'Raywood'[x]	10	5	1	4	1	17
North American						
F. americana (unnamed cultivar)[x]	6	4	2	0	2	33
F. americana cv. 'Autumn Applause'[w]	8	6	2	0	2	25
F. americana cv. 'Autumn Purple'[x]	7	2	5	0	4	71
F. americana cv. 'Rosehill'[w]	9	2	7	0	6	78
F. latifolia (unnamed cultivar)[x]	9	6	1	2	0	14
F. nigra (unnamed cultivar)[x]	9	3	4	2	4	57
F. nigra cv. 'Fall Gold'[x]	7	6	0	1	0	0
F. pennsylvanica (seedling)[z,v]	12	7	2	3	0	22
F. pennsylvanica cv. 'Georgia Gem'[x]	8	8	0	0	0	0
F. pennsylvanica cv. 'Marshall Seedless'[x]	9	8	1	0	1	11
F. pennsylvanica cv. 'Patmore'[x]	9	6	2	1	2	25
F. pennsylvanica cv. 'Summit'[x]	6	4	1	1	1	20
F. quadrangulata (unnamed cultivar)[x]	10	0	8	2	0	100
Total	*156*	*74*	*62*	*20*	*30*	

[z] Obtained from Lawyer Nursery Inc. (Plains, Montana, U.S.).
[y] Obtained from Sheridan Nurseries (Ontario, Canada).
[x] Obtained from J. Frank Schmidt & Sons Co. (Boring, Oregon, U.S.).
[w] Obtained from North Branch Nursery (Pemberville, Ohio, U.S.).
[v] Obtained from ODNR Division of Forestry Nursery (Zanesville, Ohio, U.S.).

origin, including *F. excelsior* cv. 'Golden Desert' and the two narrowleaf ash cultivars, exhibited differing survival rates, with 'Golden Desert' ash showing increased survival (71%) compared to 'Desert' ash (43%) and 'Raywood' ash (17%).

IMPLICATIONS

Every North American ash that has been screened for resistance so far has been found to be susceptible to EAB infestation. However, the degree to which they are susceptible is variable across ash species. The results of the current study closely align with what is generally being reported about the variation in susceptibility of North American ashes at present, with black ash at the one end of the spectrum, as the most susceptible, and blue ash at the other end, as the least susceptible (Herms and McCullough 2014; Herms 2015; Poland et al. 2015). In general, ashes with European origin were also found to be susceptible, although their survival rates were higher relative to the North American species in the current study. Herms (2015) found the same European species and cultivars to be just as susceptible to EAB as the most susceptible North American taxa. Other studies have reported that European ash (*F. excelsior*) is experiencing high mortality in Eastern Europe, where EAB has also established and is spreading (Orlova-Bienkowskaja 2014). Because this study was conducted with fewer replicates of European ash cultivars, it is suggested that further investigation be done to assess the susceptibility of these cultivars. As in previous studies (reviewed by Villari et al. 2016), the Manchurian ash cultivar 'Mancana' was found to be resistant.This resistance was also observed across all 10 of the Manchurian ash seedlings, suggesting that EAB-resistance may be distributed across the *F. mandshurica* species and wasn't just a rare occurrence randomly captured in the cultivar (Herms 2015).

Although the study reaffirmed what is generally reported in the area of interspecific variation

in ash resistance to EAB, it also revealed some key findings that could potentially have some important implications, which are discussed below.

Importance of 'Northern Treasure' Ash

The higher survival rate of 'Northern Treasure' ash at end of the 12-year monitoring period corroborates the more recent reports of its relative resistance (Herms 2015). The original cross between black ash and Manchurian ash was an attempt to combine the cold hardiness trait of the female black ash with the growth rate and form of the male Manchurian ash (Davidson 1999). This commercially available hybrid was previously evaluated for EAB resistance and was reported to be susceptible (Rebek et al. 2008). It is possible that confusion and mislabeling in nursery production may have occurred between 'Northern Treasure' and black ash (D.A. Herms, The Ohio State University, personal communication) in the study by Rebek et al. (2008). On the contrary, an increased survival of 'Northern Treasure' ashes observed in the current study could possibly be because of the passing of the initial wave of EAB infestation through the plantation and the corresponding shrinkage in the density of EAB population in the area leaving only host preference as the primary mechanism of defense against EAB. This could mean that eventually these trees will die. Nevertheless, this finding, and that of Herms (2015), is in clear contrast to the finding reported earlier by Rebek et al. (2008) in which these hybrid trees were reported to have had no resistance whatsoever and had not inherited any resistant trait from the resistant parent Manchurian ash. This suggests that an inclusion of this hybrid ash could prove beneficial in future host-preference studies. To resolve any taxonomic confusion, further testing of carefully validated 'Northern Treasure' ash is warranted, as is further investigation of other Manchurian × black ash hybrids, including 'Northern Gem'. Demonstrating that EAB resistance may be inherited in these hybrids is an important first step to increasing resistance in North American ash through a hybridization program similar to the America Chestnut Foundation's

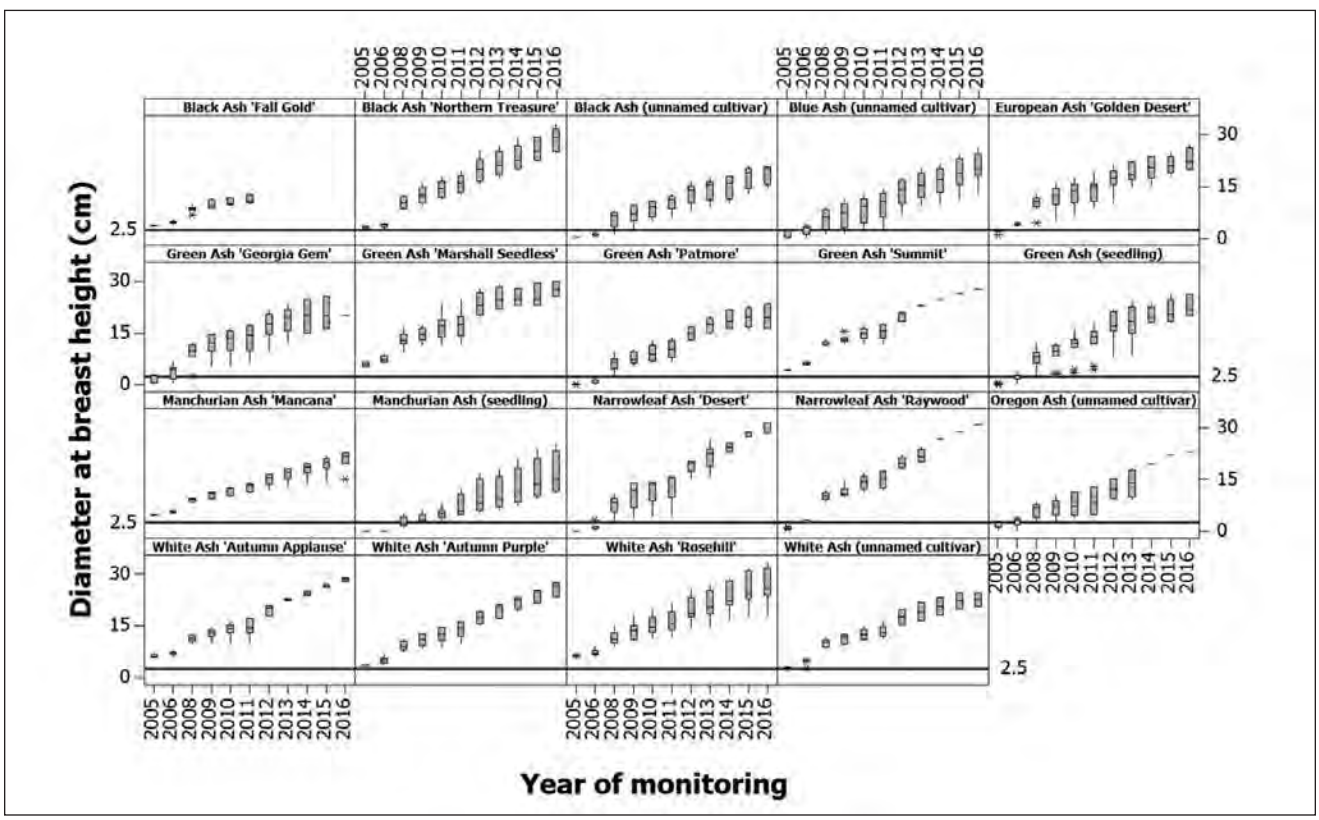

Figure 1. Diameter at breast height for the 17 cultivars and 2 seedlings measured throughout the monitoring period between 2005 and 2016. The reference line is drawn at 2.5 cm, which is generally reported as the size at which ash trees are infestable by EAB.

blight resistance breeding program. Evaluation of this cultivar is especially important because black ash is the only North American ash closely related to an Asian ash under current phylogeny (Wallander 2008), and has already been shown to cross-hybridize readily with Manchurian ash.

Cultivar Differences

To date, the reports on the susceptibility of white ash species to EAB are somewhat mixed. Rebek et al. (2008), in a garden experiment, reported a survival rate of 25% for white ash cultivar 'Autumn Purple' at the end of a three-year monitoring period, and they concluded that any variation observed in the resistance was of little practical significance. Cipolloni et al. (2011), studying the phenolic chemistry of white ash cultivar 'Autumn Purple', concluded that this cultivar has very low levels of defense traits and is hence susceptible to EAB. Klooster et al. (2014), reporting on the susceptibility of white ash, highlighted that EAB has killed 99% of the white ash in southeastern Michigan forests. More recently, however, Tanis and McCullough (2015) suggested that white ash cultivar 'Autumn Purple' may be a less preferred host. In the current study, white ash cultivars 'Autumn Purple' and 'Rosehill' are showing intermediate survival rates.

The expression of inter-cultivar variation in host-plant resistance is not something new in forest pest management. For example, the American elm cultivar 'Princeton', which was initially selected in 1922 for its vigorous growth and some degree of resistance to elm leaf beetle, was later found to be highly tolerant to the Dutch elm disease fungus (Santamour and Bentz 1995). Any noticeable inter-cultivar variation in the white ash species could have implications in ash tree management, given the preferential planting of the cultivars 'Autumn Purple' and 'Rosehill' as the third and fourth most popular white ash cultivars in urban areas (Nowak and Sydnor 1992). The current study was carried out with a small sample size of the white ash cultivars 'Autumn Purple' and 'Rosehill'. Furthermore, for both cultivars, all but one surviving individual were infested at the end of the study. Hence, it is imperative that further research be carried out to confirm these implications.

Similar to white ash, researchers also observed differences in survival rates of the two black ash cultivars ('Fall Gold' and an 'unnamed cultivar'), suggesting that there might be some possible differences in the degree to which they are resistant. Black ash is generally reported as the most susceptible of all the North American species (Herms 2015; Poland et al. 2015). Klooster et al. (2014) documented 100% mortality of black ash across an extensive series of monitoring plots in southeastern Michigan forests. Even though the 'unnamed cultivar' in the current study showed lower mortality, further screening is needed to make any interpretations about this cultivar as well, because only four trees (out of seven) survived, and all of them were infested with EAB.

Blue Ash: A Less Preferred Host

In 2004, in a common garden study established at Michigan State University's Tollgate Education Center (Novi, Michigan), Herms (2015) observed that blue ash had the highest survival (65% in 2014), of the North American taxa evaluated, but lower survival and greater canopy decline than Manchurian and 'Northern Treasure' ash. According to Herms (2015), the decline of blue ash is advancing over time, suggesting that blue ash mortality may continue to increase. From a survey of ash trees in two woodlot sites in southeastern Michigan in 2004–2005, Anulewicz et al. (2007) anticipated that all blue ash would eventually die after the death of the preferred white ash. However, seven years later, at the same two sites, Tanis and McCullough (2012) reported a surprising survival of large numbers of blue ash trees (67% on average across the two sites). The relative resistance of blue ash was also reported by Tanis and McCullough (2015) in a subsequent study. Peterson et al. (2015) found that larval EAB performance on blue ash and highly susceptible green ash did not differ, which suggests that resistance of blue ash is due to low oviposition preference. The higher survival rates of blue ash observed in the current study further affirms claims that blue ash is a less preferred host among the North American ash taxa. The finding underscores the need for evaluating resistance mechanisms in blue ash, including oviposition preference of EAB for blue ash.

CONCLUSION

A smaller sample size and the limited spatial extent may somewhat limit the extent to which researchers may regard the interpretations made from this study. Nevertheless, the observations themselves, along with the temporal extent of the study, provide some useful insights for EAB-ash screening and host-preference studies in the near future. While commenting on the importance of screening studies, Herms (2002) highlighted their value stemming from the cost effectiveness of such studies. Hence, a thorough screening of existing ash genotypes is recommended for identifying host resistance.

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Résumé. Identifier les génotypes du frêne résistants à l'agrile du frêne (*Agrilus planipennis* Fairmaire) (AF) représente toujours un effort d'importance afin d'accroître notre connaissance de la variation interspécifique dans la préférence manifestée par l'AF en tant que plante hôte, d'identifier les mécanismes en cause dans les infestations de l'AF et d'aider au développement d'hybrides de frênes résistants à l'AF, pouvant potentiellement être utilisés pour leur remplacement dans les plantations urbaines. Une plantation pilote constituée de 17 cultivars et de deux plantules de frênes réguliers fut établie à Toleco, Ohio, USA, afin suivre de près la susceptibilité de ces arbres à l'AF. La mortalité des arbres fut compilée sur une base annuelle depuis 2005. Les résultats de l'étude et ses implications au terme de 12 années de suivi sont présentés dans cet article. L'étude a reconfirmée ce qui est connu comme étant le résistance des espèces de frênes asiatiques, *Fraxinus mandshurica* ('Mancana') et la susceptibilité générale des espèces nord américaines à l'AF. Cependant, il fut observé que le degré de susceptibilité à l'AF variait selon les divers cultivars. Ainsi, les cultivars du frêne blanc, 'Autumn Purple' and 'Rosehill', montraient une taux de survie plus élevé. Cette conclusion est cependant basée sur une faible échantillonnage et tous les frênes de chacun des cultivars étaient infestée à la fin de l'étude. Les conclusions suggèrent la nécessité de poursuivre de manière minutieuse le filtrage des cultivars de frêne afin d'identifier les génotypes résistants.

Zusammenfassung. Die Identifizierung von Eschengenotypen, die gegenüber dem Eschenprachtkäfer (*Agrilus planipennis* Fairmaire) (EAB) resistent sind, bleibt weiterhin ein wichtiger Ansatz, das bestehende Wissen über die interspezifischen Variationen in der EAB-Wirtspräferenz zu erweitern, die in die EAB-Infektion involvierten Mechanismen zu identifizieren und bei der Entwicklung von EAB-resistenten Eschenhybriden, die potentiell als überlebensfähige Ersatzpflanzungen in den urbanen Pflanzungen dienen können, zu helfen. In Toledo, Ohio, USA, wurde eine Forschungspflanzung, bestehend aus 17 Eschen-Kultivaren und 2 Eschensämlingen, etabliert, um die Anfälligkeit dieser Bäume gegenüber EAB zu überwachen. Die Baumsterblichkeit wurde auf einer jährlichen Basis seit 2005 aufgezeichnet. Am Ende der 12 jährigen Überwachung wurden die Resultate aus dieser Studie und ihre Implikationen in diesem Bericht präsentiert. Diese Studie bestätigt, was bereits über die Resistenz von der Asiatischen Eschenart *Fraxinus mandshurica* ('Mancana') und der generellen Anfälligkeit der Nordamerikanischen Eschen gegenüber EAB bekannt ist. Dennoch wurde auch beobachtet, dass der Grad der Anfälligkeit gegenüber EAB unter den verschiedenen Kultivaren variierte, wobei die Kultivare der Weissen Esche 'Autumn Purple' und 'Rosehill' höhere Überlebensraten zeigten. Dennoch basiert dieses Ergebnis auf einer niedrigen Replikation, und alle bis auf ein Individuum jedes Kultivars war am Ende der Studie infiziert. Die Ergebnisse verdeutlichen einen Bedarf an fortfahrenden und gründlichen Forschungen an Eschen-Kultivaren, um resistente Genotypen zu identifizieren.

Resumen. La identificación de genotipos de fresno resistentes al barrenador esmeralda (*Agrilus planipennis* Fairmaire) (BEF) sigue siendo un esfuerzo de importancia para ampliar el conocimiento sobre la variación interespecífica en la preferencia de EAB-huésped, identificar los mecanismos implicados en la infestación de EAB y ayudar al desarrollo de híbridos de fresno resistentes a EAB que podrían servir como reemplazo viable en plantaciones urbanas. Se estableció una plantación de investigación compuesta por 17 cultivares y 2 brinzales de árboles de fresno en Toledo, Ohio, EE. UU., para monitorear la susceptibilidad de estos árboles al BEF. La mortalidad del árbol se registró anualmente desde 2005. Los resultados del estudio y sus implicaciones, al final de los 12 años de seguimiento, se presentan en este documento. El estudio reafirmó lo que se sabe sobre la resistencia de la especie de fresno asiático *Fraxinus mandshurica* ('Mancana') y la susceptibilidad general de los fresnos de América del Norte a BEF. Sin embargo, se observó que el grado de susceptibilidad al EAB variaba entre los diferentes cultivares, y los cultivares de fresno blanco 'Autumn Purple' y 'Rosehill' mostraban mayores tasas de supervivencia. Aun así, esta conclusión se basa en una baja replicación, y todos menos uno de cada cultivar fueron infestados al final del estudio. Los hallazgos sugieren la necesidad de una revisión continua y exhaustiva de los cultivares de fresno para identificar genotipos resistentes.