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Non-native Pest

Biology and Control of Emerald Ash Borer

Edited by Roy G. Van Driesche and Richard C. Reardon

On the cover: Cover design by Sheryl Romero and Denise Binion, Forest Health Technology Enterprise Team. Background image: Understory green ash seedlings (*Fraxinus pennsylvanica*, Oleaceae) released after large ash trees were killed by emerald ash borer in Okemos, Michigan in 2014, photo by Leah S. Bauer; (bottom row, left to right) Fully mature *Tetrastrichus planipennisi* larvae break free of emerald ash borer larval skin and pupate in the larval gallery under the tree bark. (Photo credit: Clifford Sadof); EAB adult and typical leaf feeding damage. (Photo credit: Deborah Miller, USDA Forest Service, Bugwood.org); Emerging *Tetrastrichus plannipennisi* adults. (Photo credit Leah S. Bauer).

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TABLE OF CONTENTS

Chapter 1: Emerald Ash Borer Biology and Invasion History

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INTRODUCTION

The emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is native to eastern Asia and is primarily a pest of ash (*Fraxinus*) trees (Fig. 1). Established populations of EAB were first detected in the United States and Canada in 2002 (Haack et al., 2002), and based on a dendrochronology study by Siegert et al. (2009), the original EAB introduction likely occurred in the early to mid-1990s in Michigan. In European Russia, EAB was first found near Moscow in 2003, but not officially identified until 2005 (Izhevskii and Mozolevskaya, 2010). EAB has become a serious pest of ash in North America and European Russia, is causing widespread tree mortality, and is spreading rapidly on both continents (Cappaert et al., 2005; Poland and McCullough, 2006; Kovacs et al., 2010; Baranchikov, 2013; EAB Info, 2013; Orlova-Bienkowskaja, 2013; Straw et al., 2013; Herms and McCullough, 2014). In this chapter, we discuss the biology of EAB, its native and introduced range through 2013, and the likely pathways by which it was introduced and spread.

General Biology

The life cycle of EAB is typically completed in one year, but two years is often required, especially in vigorous hosts, in cooler climates, or when eggs are laid late in the season (Cappaert et al., 2005; Wei et al., 2007; Wang et al., 2010). In addition, Petrice and Haack (2007) reported that EAB may require two years to complete development in cut logs or firewood, especially when the wood has dried. EAB can successfully infest both healthy and stressed ash

Figure 1. Adult emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae). (Photo credit: David Cappaert, Michigan State University, Bugwood.org)

trees in North America and European Russia, where the native ash species did not coevolve with EAB (Liu et al., 2003; Wei et al., 2004; Poland and McCullough, 2006; Baranchikov et al., 2008; Rebek et al., 2008). However, within EAB's native range in China and the Russian Far East, species of Asian ash are usually resistant to the borer, except during periods of environmental stress such as prolonged drought (Yu, 1992; Zhao et al., 2005; Baranchikov et al., 2008). In addition, EAB has been reported to kill species of North American ash that were planted in China and Russia (Liu et al., 2003; Zhao et al., 2005; Baranchikov et al., 2008). EAB infests nearly all sizes of ash trees, from saplings that measure 2-3 cm in diameter to mature trees (Haack et al., 2002; Wei et al., 2007), and infests both open-grown and interior-forest trees (Poland and McCullough, 2006; McCullough et al., 2009; Wang et al., 2010) (Figs. 2-3).

In China and the Russian Far East, the principal native hosts of EAB include *Fraxinus mandshurica* Ruprecht and *Fraxinus chinensis* Roxburgh (Yu, 1992; Zhao et al., 2005; Wei et al., 2007; Baranchikov et al.,

2008; Izhevskii and Mozolevskaya, 2010), whereas in North America, EAB has been able to infest and kill all species of native *Fraxinus* so far encountered, including *F. americana* L., *F. nigra* Marshall, *F. pennsylvanica* Marshall, *F. profunda* (Bush) Bush, and *F. quadrangulata* Michx. (Anulewicz et al., 2008; EPPO, 2013). In European Russia, EAB has infested and killed primarily the introduced North American species *F. pennsylvanica* and the native European species *Fraxinus excelsior* L. (Baranchikov et al., 2008; Izhevskii and Mozolevskaya, 2010; Duan et al., 2012). It is important to note that Jendek (1994) synonymized two other Asian *Agrilus* species and one subspecies with *A. planipennis*, type China (EAB), including *Agrilus feretrius* Obenberger (type Taiwan), *Agrilus marcopoli* Obenberger (type Mongolia), and *Agrilus marcopoli* ulmi Kurosawa (type Japan) (Jendek and Grebennikov, 2011). Besides ash, which is the only larval host reported for *A. planipennis* in China (Yu, 1992; Liu et al., 2003; Zhao et al., 2005), other tree genera (*Juglans*, *Pterocarya*, and *Ulmus*) were reported as larval hosts in Korea and Japan for A. *marcopoli* and A. *marcopoli ulmi* (Ko, 1969; Akiyama and Ohmomo, 1997). However, in a recent EAB pest risk assessment prepared by the European and Mediterranean Plant Protection Organization (EPPO, 2013), it was reported that Japanese buprestid specialists now consider the non-*Fraxinus* host records for Japan to be in error.

The adult flight season of EAB usually begins in May or June in the Great Lakes region of North America and at similar latitudes in Asia, with peak flight occurring in June to July, and usually ending by September (Cappaert et al., 2005; Wei et al., 2007; Wang et al., 2010). Adults are most active on sunny days when air temperatures exceed 25° C (Wang et al., 2010), but during rainy or cool weather the adults often rest in bark crevices and on leaves (Rodriguez-Saona et al., 2007). EAB adults consume host foliage throughout their life and can live for several weeks under favorable laboratory conditions (Fig. 4; Wang et al., 2010; EPPO, 2013).

EAB adults use visual and olfactory cues to locate host trees and mates. Shades of purple and green are highly attractive to EAB adults (Francese et al., 2005, 2008, 2010; Crook et al., 2009). Moreover, EAB

Figure 2. Recently planted ash trees showing thinning crowns and epicormic shoots typical of EAB infestation. (Photo credit: Leah Bauer, USDA Forest Service, Bugwood.org)

Figure 3. Mature ash tree showing dieback typical of EAB infestation and bark removal by woodpeckers as they search for EAB life stages. (Photo credit: Steven Katovich, USDA Forest Service, Bugwood.org)

adults are attracted to dead EAB adults when placed on foliage or traps as decoys (Lelito et al., 2007, 2008; Petrice et al., 2013). This is not surprising given that EAB adult males are known to hover near host trees when searching for mates, and then landing on or near prospective mates when they are located (Lelito et al., 2007; Rodriguez-Saona et al., 2007). Various volatiles from ash bark and foliage elicit positive responses in EAB adults under laboratory conditions, and some of these compounds increase EAB attraction to purple or green traps (Rodriguez-Saona et al., 2006; Crook et al., 2008; Crook and Mastro, 2010; Grant et al., 2011; Poland et al., 2011; Poland and McCullough, 2014). Also, close range sex pheromones have been identified for EAB, and field testing has found them to increase attraction of EAB to traps (Lelito et al., 2009; Silk et al., 2011; Ryall et al., 2012).

EAB adults mate on the trunk, branches, and foliage of their host plants. Oviposition usually begins about 5-10 days after adult emergence. Eggs are laid individually or in small clusters between layers of bark and in bark crevices along the trunk, major branches, and exposed roots (Wei et al., 2007; Anulewicz et al., 2008; Wang et al., 2010; Jennings et al., 2014) (Fig. 5). Under laboratory conditions, average adult female longevity is about 7-9 weeks, with total fecundity usually averaging between 40 to 74 eggs per female and with a maximum of 307 eggs (Rutledge and Keena, 2012; Jennings et al., 2014). Average EAB adult male longevity is about 43 days (EPPO, 2013).

Egg hatch usually occurs after 7-18 days, depending on local temperatures. Neonate larvae chew through the surface of the egg that is in contact with the tree, and tunnel directly through the outer bark to the cambial region where they feed on the inner bark (phloem) and outer sapwood, creating frass-filled galleries (Wei et al., 2007; Wang et al., 2010). Larval galleries tend to be more serpentine-shaped in vigorous host trees (Fig. 6), while more meandering in less vigorous hosts or when larval densities are high (Wei et al., 2007; Wang et al., 2010) (Fig. 7).

Chamorro et al. (2012) described the morphology of EAB eggs, larvae, and pupae in great detail. Briefly, EAB has four larval instars, and as is typical of larvae in the genus *Agrilus*, there are two heavily sclerotized processes, often called urogomphi, at the terminal

Figure 4. Typical leaf feeding damage by EAB adults. (Photo credit: Deborah Miller, USDA Forest Service, Bugwood.org)

Figure 5. EAB eggs are white in color when first deposited (a) and then turn yellowish-brown within a few days (b). (Photo credit: Houping Liu, Michigan State University, Bugwood.org)

end of the abdomen (Fig. 8). Measurements of the urogomphi can be used to distinguish the larval instars (Liu et al., 2007; Petrice et al., 2009; Wang et al., 2010). For individuals that complete their life cycle in one year, larvae overwinter as mature fourth instars (Cappaert et al., 2005; Wang et al., 2005, 2010). For individuals that develop over two years, the first winter is usually spent as early instar larvae. Once a larva completes its feeding as a fourth instar (Fig. 9) it constructs a pupal cell, usually in the outer sapwood of thin-barked branches or trees or in the outer bark of thick-barked trees (Abell et al., 2012). Before creating the pupal cell, 4th-instar larvae construct a tunnel that extends nearly to the surface of the outer bark that will later be used by the new adult when it exits the tree (Wang et al., 2010). In the newly

completed pupal cell, the 4th-instar larva folds itself into a J-shape or U-shape before overwintering (Fig. 10).

In spring, the larvae that overwintered in pupal cells develop into prepupae by gradually unfolding their body as they become shorter and more cylindrical. Prepupae then molt into naked or exarate pupae (Wang et al., 2010). Pupation occurs in late spring and early summer and usually lasts 3-4 weeks (Fig. 11). After eclosion, the newly formed or pharate adult will remain in its pupal cell for about one week before it chews its way out of the tree by enlarging the exit tunnel that it created earlier when it was a mature larva (Wei et al., 2007; Wang et al., 2010). The exit hole constructed by the adults is typically D-shaped, with the flat side of the "D" corresponding

Figure 6. EAB larval gallery in a vigorous host as evidenced by the tight zig-zag pattern of the gallery and the attempt by the tree to compartmentalize the gallery. (Photo credit: Edward Czerwinski, Ontario Ministry of Natural Resources, Bugwood)

Figure 8. Close-up of the paired terminal processes found at the tip of the last abdominal segment of EAB larvae. (Photo credit: Pennsylvania Department of Conservation and Natural Resources, Bugwood.org)

Figure 7. EAB larval galleries on a less-vigorous host tree as evidenced by the meandering pattern of the galleries. (Photo credit: Edward Czerwinski, Ontario Ministry of Natural Resources, Bugwood.org)

Figure 9. Fourth instar EAB in its gallery, which is constructed in the cambial region of the tree. (Photo credit: Pennsylvania Department of Conservation and Natural Resources, Bugwood.org)

to the upper side of the adult's body (Fig. 12). Upon emergence, adults readily walk or fly to host foliage and feed (Wang et al., 2010).

As is typical for many *Agrilus* species, trees often die after 1-3 years of successive borer infestation, with death usually beginning in the crown branches and moving downward in subsequent years to the main trunk (Haack and Benjamin, 1982; Cappaert et al., 2005; Ryall et al., 2011; Foelker et al., 2013). However, in small diameter ash trees, initial EAB infestations often begin on the main trunk (Timms et al., 2006; Wei et al., 2007; Tluczek et al., 2011). In many EAB-infested ash trees, epicormic branches develop along the lower trunk before the tree dies (Cappaert et al., 2005; Wang et al., 2010) (Figs. 2, 13).

Native Range of Emerald Ash Borer

EAB is native to China, Japan, Korea, Mongolia, the Russian Far-East, and Taiwan (Yu, 1992; Jendek, 1994; Jendek and Grebennikov, 2011; Chamorro et al., 2014). The recent report of EAB from Laos (Jendek and Grebennikov, 2011) is no longer considered valid given that the specimens examined from Laos were later described as a new species: *Agrilus tomentipennis* (Jendek and Chamorro, 2012). Although EPPO (2013) raised doubts on the occurrence of EAB in Mongolia, Jendek and Grebennikov (2011) state that the type specimen for A. *marcopoli* is from Mongolia. In addition, as stated above, the occurrence of EAB in Japan and Taiwan is based on specimens that were formerly considered A. *marcopoli ulmi* and A. *feretrius*, respectively (Jendek, 1994; Jendek and Grebennikov, 2011).

INITIAL DISCOVERY AND SPREAD OF EAB IN NORTH AMERICA

EAB was first discovered in North America in 2002 (Haack et al., 2002; Cappaert et al., 2005; Poland and McCullough, 2006; Herms and McCullough, 2014). The first adults were reared from declining ash trees near Detroit, Michigan, in May 2002, and were sent to several taxonomists for identification. Later, in July 2002, they were positively identified as *A. planipennis* by Eduard Jendek in Slovakia, who is the world

Figure 10. Fourth instar EAB larvae in their typical overwintering position (J-larvae) inside pupal cells that were constructed in the outer sapwood. (Photo credit: Houping Liu, Michigan State University, Bugwood.org)

Figure 11. EAB pupae are naked and gradually mature into adults within their individual pupal cells during spring and summer. (Photo credit: Deborah Miller, USDA Forest Service)

authority on Asian *Agrilus*. Moreover, beetles that looked similar to EAB were collected in July 2002 in Windsor, Ontario, and identified as EAB in August 2002.

Michigan and Canada enacted quarantines on all known EAB-infested counties starting in July and September 2002, respectively (Haack et al., 2002). A federal EAB quarantine was first enacted

in the United States in October 2003 (USDA APHIS, 2003). The EAB quarantine zone has expanded each year since 2002 in both the United States and Canada as a result of regional surveys in several states and provinces. When new EAB populations were discovered, quarantines were usually enacted at the county level. As a result of intense survey efforts, the steady range expansion of EAB has been well documented in North America (Fig. 14). However, it is important to realize that it usually takes several years before EAB populations are large enough to be detected during surveys. Range expansion of EAB is a result of both natural spread and artificial movement of infested ash material.

By the end of 2002, EAB had been found in six southeastern Michigan counties in the Detroit area. This number increased to 13 counties by the end of 2003, and 20 by 2004, all still within Michigan's Lower Peninsula. In 2005, EAB was found for the first time in Michigan's Upper Peninsula. EAB was first found in Ohio in 2003; Indiana in 2004; Illinois and Maryland in 2006; Pennsylvania and West Virginia in 2007; Wisconsin, Missouri and Virginia in 2008; Minnesota, New York, and Kentucky in 2009; Iowa and Tennessee in 2010; Connecticut, Kansas, and Massachusetts in 2012; and Colorado, Georgia, New Hampshire, and North Carolina in 2013 (EAB Info, 2013) (Fig. 14). In Canada, EAB has been detected in just two provinces as of 2013, first in Ontario in 2002, and then in Quebec in 2008 (Fig. 14). Overall, as of December 2013, EAB was known to occur in 22 U.S. states and two Canadian provinces.

INTRODUCED RANGE AND SPREAD OF EAB IN RUSSIA

There are few early records of EAB from Russia. During the 1900s, all EAB records were from southern Primorskiy Kray in the Russian Far East and consisted of small numbers of specimens collected during 1935-1999 (Alekseyev, 1979; Jendek, 1994; Volkovich, 2007; Yurchenko et al., 2007; Fig. 15). In 2004, EAB populations were also found in southern Khabarovsk Kray in the Russian Far East in the area between Khabarovsk and Dzonki, a distance of about 100 km as measured along the Amur River

Figure 12. EAB adults construct D-shaped exit holes as they chew through the bark and emerge from their host tree. (Photo credit: Deborah Miller, USDA Forest Service)

Figure 13. EAB infested ash tree with epicormic shoots that often develop during the latter years of infestation prior to tree death. (Photo credit: Edward Czerwinski, Ontario Ministry of Natural Resources, Bugwood.org)
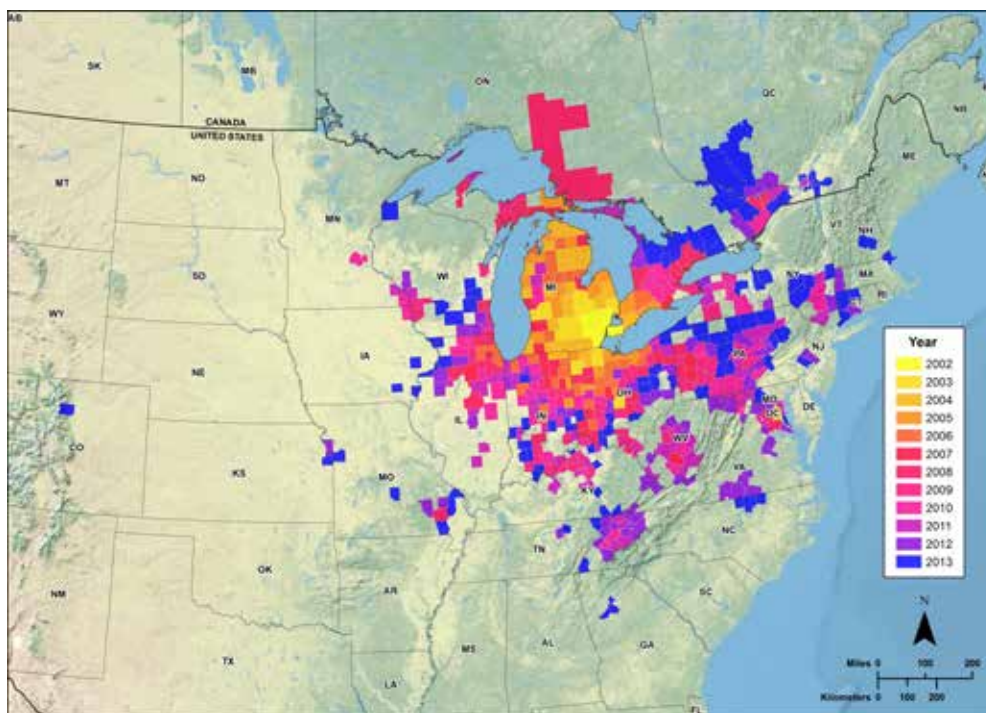
Figure 14. (top) Annual spread of EAB in North America from 2002 through 2013 as determined by year of first detection. Service layer credits: US National Park Service. Data sources: USDA Animal & Plant Health Inspection Service (APHIS), Canadian Food Inspection Agency (CFIA). Map developed by USDA Forest Service, Northeastern Area State and Private Forestry, Office of Knowledge Management (T. Luther 04/15/2014).
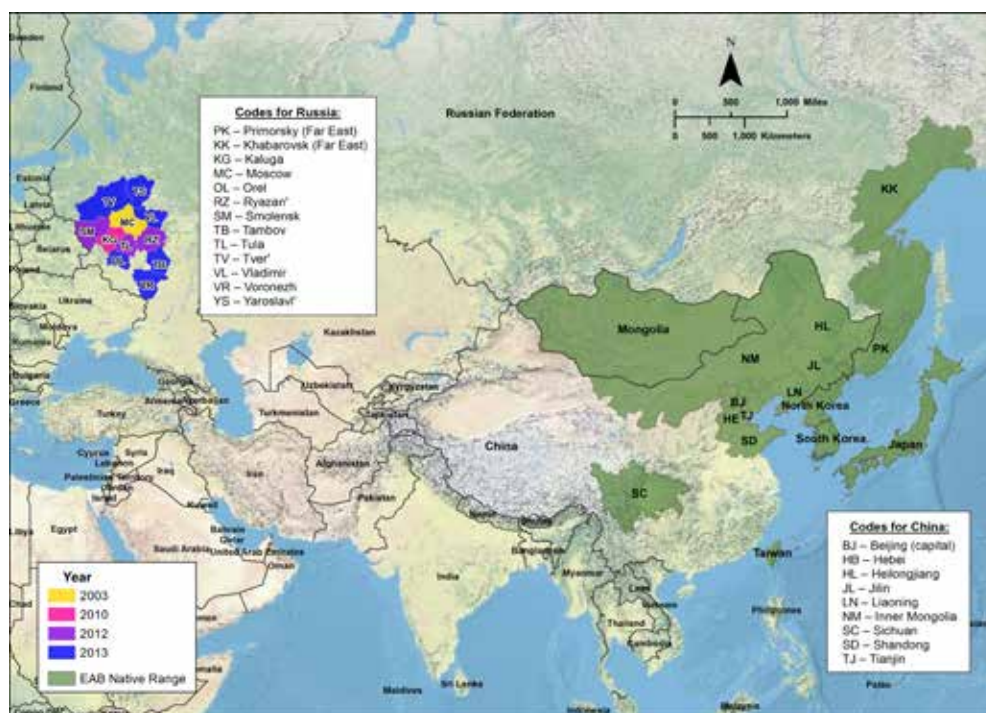
Figure 15. (bottom) Known range of EAB in Asia and introduced area of EAB in European Russia as of 2013. Service layer credits: US National Park Service. Data sources: https://sites.google.com/site/eduardjendek/world-distribution-of-agrilus-plannipennis_ and Baranchikov (2013). Map developed by USDA Forest Service, Northeastern Area State and Private Foresty, Office of Knowledge Management (T. Luther 04/15/2014).

(Yurchenko, 2010; Fig. 15).

Historically, EAB was a rare species in the Russian Far East, where it was associated exclusively with weakened and dying local native ash trees such as *F. mandshurica* and *F. chinensis*. Widespread tree mortality associated with EAB was first noticed in the Russian Far East in 2004, affecting introduced North American ash trees (*F. pennsylvanica*) that were growing along streets in Vladivostok (Yurchenko, 2010), many of which were rather mature trees with trunks measuring 20-40 cm in diameter. Subsequent detailed investigations of dead North American ash trees (*F. americana* and *F. pennsylvanica*) in parks and arboreta in Khabarovsk demonstrated that these trees had been killed by EAB during the previous 5-10 years when the trees were 28-35 years old (Yurchenko, 2010).

In European Russia, beetles that were later to be identified as EAB were first collected on the streets of Moscow in June 2003 (Fig. 15; Volkovich, 2007). These beetles were positively identified as *A. planipennis* in 2005 by A. B. Alekseyev – the leading Russian expert on Buprestidae (Izhevskii and Mozolevskaya, 2010). It was soon recognized that EAB was responsible for the widespread ash dieback in Moscow (Baranchikov et al., 2008; Mozolevskaya et al., 2008). From 2006-2013, EAB spread outward from Moscow (Fig. 15). In 2006, 10 EAB adults were collected 30 km west of the Moscow Ring Highway (Volkovich, 2007), and by 2009, EAB-killed ash trees were found in many settlements of the Moscow Oblast region, with the most westward known infestation in Mozhaisk, about 100 km from Moscow (Baranchikov et al., 2010a). EAB was found in the Kaluga Region in 2010, and in the Smolensk and Ryazan Regions in 2012 (Baranchikov and Kurteyev, 2012; Baranchikov, 2013). Similarly, in 2013, EAB was first reported in the Orel, Tambov, Tula, Tver, Vladimir, Voronezh, and Yaroslavl Regions (Baranchikov, 2013, Orlova-Bienkovskaya, 2013). The current known range of EAB in European Russia is close to the borders of Belarus and Ukraine (Fig. 15).

Special EAB surveys were conducted during 2008-2009 on *F. pennsylvanica* in several cities throughout southern Siberia (Abakan, Krasnoyarsk, Novosibirsk, Tomsk, and Ulan-Ude) and the central Urals (Yekaterinburg). However, no additional EAB populations were found during these surveys (Baranchikov et al., 2010b).

PATHWAYS OF EMERALD ASH BORER DISPERSAL

It is not known for certain how EAB reached North America or European Russia. In North America, wood packaging material such as pallets and crating from Asia is considered the most likely source (Haack et al., 2002, Cappaert et al., 2005, Haack, 2006). Recent genetic analyses by Bray et al. (2011) and Keever et al. (2013) found that North American EAB populations were most similar to Chinese populations, less so with Korean populations, and least similar to Japanese populations. As for European Russia, Izhevskii and Mozolevskaya (2010) suggested that EAB could have been introduced on ash nursery stock imported from North America or on wood packaging material from Asia. Genetic analyses may not help determine the source of the Moscow EAB population because molecular testing has shown high similarity among North American, Chinese, Far-East Russian, and Moscow EAB populations (EPPO 2013).

In North America and Europe, EAB can spread naturally through adult flight, which can expand the infested area by several kilometers each year (Taylor et al., 2010; EPPO, 2013). However, EAB dispersal over distances of 10s or 100s of kilometers most likely results from human movement of infested host material such as ash nursery stock, logs, and firewood (Cappaert et al., 2005; Haack, 2006; Poland and McCullough, 2006; Haack et al., 2010; Herms and McCullough, 2014). For example, a nursery in Michigan sold EAB-infested nursery stock to a Maryland nursery in 2003, which then sold some of the trees to individuals living in Maryland and Virginia before realizing the trees were infested (Muirhead et al., 2006). Similarly, a sawmill near Shipshewana, Indiana, which regularly purchased ash sawlogs from southern Michigan was apparently responsible for introducing EAB to the local area (Robertson and Andow, 2009). Firewood is believed to be a major pathway by which EAB

has been introduced to many residential areas, vacation properties, and campgrounds (McCullough et al., 2003; Robertson and Andow, 2009; Haack et al., 2010). In addition, EAB adults have been documented to hitchhike on or inside vehicles, as well as on passengers, which may explain their high frequency of establishment along major highways, especially at rest areas and truck stops (Buck and Marshall, 2008). The means of long-distance EAB dispersal in European Russia is not clear given that movement of ash nursery stock, firewood, and logs is rare in Russia, and therefore hitchhiking by EAB adults on vehicles is considered the most likely explanation (Straw et al., 2013).

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CHAPTER 2: ECOLOGICAL IMPACTS OF EMERALD ASH BORER

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INTRODUCTION

The genus *Fraxinus* includes 16 North American species. Flowers et al. (2013) estimate that there are more than 8.7 billion ash trees and saplings in the continental United States, and these are all potentially susceptible to emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) infestation. Since its invasion, this beetle has established in more than 20 states, from Minnesota to Maine, south to Georgia and Missouri, as well as in southern Ontario and Quebec, and is expanding its range on all edges of its current distribution. Because timber and firewood from infested areas can be transported long distances, the beetle has the potential to establish virtually anywhere where ash species grow. It is considered to be among the most destructive forest insect pests to have been introduced into North America (Herms and McCullough, 2014; McCullough and Usborne, 2014). More than 200,000 million ash trees have been killed – especially in the Great Lakes region where the insect first established in the early 1990s (Cappaert et al., 2005a; Poland and McCullough, 2006, Siegert et

Figures 1-4. Affected communities in Ohio; dead trees are green ash (*F. pennsylvanica*) killed by the emerald ash borer: (1) Saint Mary's River west of Decatur, June 2014. (Photo courtesy Jim McCormac); (2) Willow Point Wildlife Area in Vickery, August 2011. (Photo courtesy Jim McCormac); (3, 4) Ottawa National Wildlife Refuge in Oak Harbor, August 2014. (Photos courtesy Judy Semroc)

al., 2007) (Figs. 1-4).

Fraxinus americana L. (white ash) and especially *Fraxinus pennsylvanica* Marshall (green ash) – strong, stately, rapidly growing trees – are widely planted in yards, parks, and along city streets, in part because both species are known to be hardy and relatively insect- and disease-free (Burns and Honkala, 1990). Ash is also preferred for firewood because its sap is flammable and, as such, the potential for EAB to be spread throughout North America by human transport of infested wood is greatly heightened. A lower bound economic estimate for treating, removing, and replacing EAB-infested ash trees in urban landscapes for 2010 to 2020 is $12.5 billion (Kovacs et al., 2011). White ash and, to a lesser extent, green ash are commercially important trees whose strong but flexible wood is used for flooring, paneling, furniture, tool handles, and baseball bats (Elias, 1987; Burns and Honkala, 1990). White ash has long been the preferred wood for the Louisville Slugger – a bat popular with professional baseball players.

Ash typically grows as a component in hardwood forests (Figs. 5-11); furthermore, *Fraxinus* species are classified as either the dominant or co-dominant species in 150 forest and shrubland communities (NatureServe Explorer, 2014, see below). In some wetlands, species such as *Fraxinus nigra* Marshall (black ash) (Figs. 8, 10) and *Fraxinus profunda* (Bush) Bush (pumpkin ash) (Figs. 7, 9) form almost pure monocultures that are highly susceptible to EAB (Tardif and Bergeron, 1992; Rebek et al., 2008; Klooster et al., 2014; NatureServe Explorer, 2014; Jim McCormac and Jim Bissell pers. comms). Laboratory trials suggest that all 16 species of North American *Fraxinus* may be suitable hosts for larval development of EAB and thus are susceptible to attack (Anulewicz et al., 2008; Leah Bauer pers. comm, Deb McCullough pers. comm). Even small-stemmed western species have the potential to support the beetle given that saplings of white and green ash as narrow as 1 cm in diameter are exploited by the beetle and its immature stages in the eastern United States. Once infested, tree death typically follows within 2 to 6 years (Knight et al., 2013). In addition, the related (olive family) fringetree (*Chionanthus virginicus* L.) is also susceptible to attack, but the beetle's impact on that host is as yet unstudied

(Entomology Today, 2014).

Climate models for the beetle and projections for its spread are still in development and burdened with considerable uncertainty (Sobek-Swant et al., 2012; Liang and Fei, 2014), thus it is impossible to know how far north, south, and west the beetle might spread in North America and which *Fraxinus* populations are most at risk. Consequently, for the purposes of this assessment, we consider the entirety of the North American ash flora to be vulnerable. We first examine the forest community types where ash is a dominant or co-dominant and then provide a brief assessment for each of the sixteen *Fraxinus* that grow north of Mexico. Each treatment includes a synopsis of a species' habitat, range, conservation status, known susceptibility to EAB, and a summary of its specialist herbivores. We then include a brief discussion of the importance of ash to vertebrate wildlife before addressing the core of our contribution – a comprehensive evaluation of the invertebrate herbivore fauna of North American *Fraxinus* likely to be threatened by the spread of EAB. Our evaluation is constructed from reviews of literature, correspondence with taxonomic authorities, and DLW's 30-year rearing program. We ignore generalist herbivores known to feed on ash, although a few oligophagous species are discussed.

Our assessment is novel in that we considered feeding records in older and derivative literature as unconfirmed, given the large number of misattributed records plaguing recent compendia and risk-assessment literature. Instead, we adopted an authority-driven approach whereby we contacted one or more active systematists or experts for taxa known to have specialist herbivores on woody plant taxa. We received information from more than 80 taxonomic authorities with first-hand knowledge of appropriate literature, species-level taxonomy, life history data, and ecological associations. Compared to previous assessments, our approach yielded a dramatically different list of potentially imperiled herbivores, essentially half of which are newly reported here. Our work includes a revised set of risk rankings for 98 species that we believe to be threatened by the emerald ash borer.
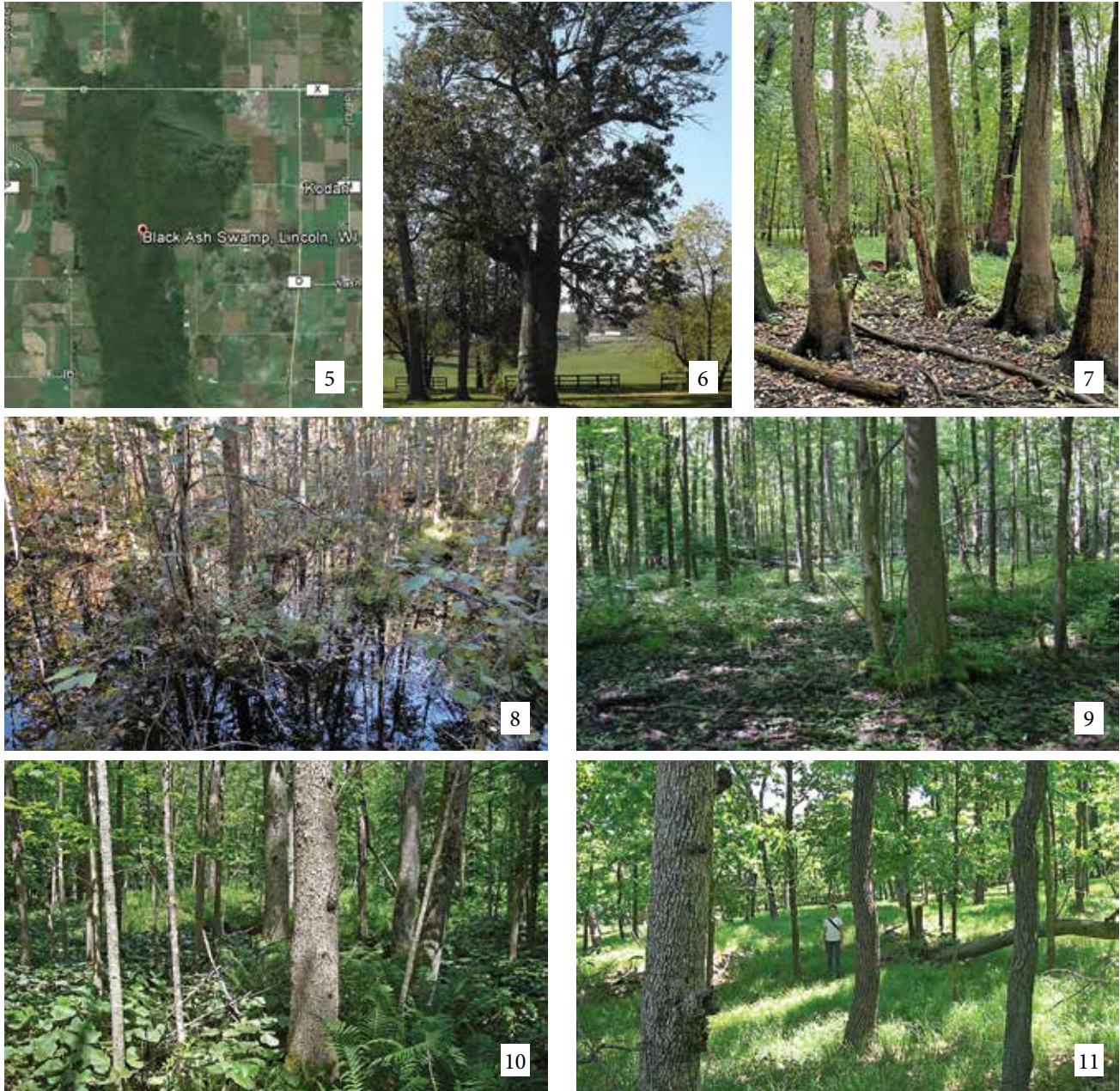
Figures 5-11. Ash trees and ash-dominated communities: (5) Google Earth view of black ash swamp west of Lincoln, Wisconsin; *Fraxinus nigra* is dominant over thousands of hectares of this boreal wetland; (6) Majestic blue ash (*F. quandrangulata*) tree near Lexington, Kentucky. (Photo courtesy Daniel Boone); (7) Vernal pool with canopy of pumpkin ash (*F. profunda*), Momence Wetlands Land and Water Reserve, Illinois; note buttress roots or knees which help the trees uptake oxygen when the pool is inundated. (Photo courtesy Judy Semroc); (8) Black ash dominated swamp near Black Lake, Michigan. (Photo courtesy Anton Reznicek); (9) Vernal pool near Astabula, Ohio with pumpkin ash as the dominant canopy tree; the dry vernal pool shown here is a breeding area for mole salamanders. (Photo courtesy Judy Semroc)—see *Effects to Vertebrates*; (10) Northern hardwood swamp dominated by black ash near Wallon Lake, Michigan. (Photo courtesy Anton Reznicek); (11) Upland hickory-white ash glade (*F. americana*) in Litchfield County, Connecticut. (Photo courtesy Ken Metzler)

METHODS AND RATIONALE

To determine ecological impacts of EAB infestations on communities, we contacted 20 people with first-hand experience (botanists, reserve managers, Department of Natural Resources (DNR) ecologists, State Nature Conservancy chapters, forest managers, land managers, seasoned naturalists, wildlife biologists, and especially those with extensive field experience) in the forests and woodlands where EAB has been resident for four or more years (Appendix 1). We focused our surveys and correspondence on three states and one province with conspicuous EAB impacts: Michigan, northern Ohio, northeastern Indiana, and southwestern Ontario. For each state or province we initially contacted a lead biologist in the DNR (or its functional equivalent) and a state or provincial office for The Nature Conservancy and then made efforts to approach additional state or provincial authorities that had been endorsed for their knowledge of the impacts of EAB. Vertebrate biologists that we contacted for information on the ecological (especially dietary) importance of *Fraxinus* are listed in Appendix 1. Conversations with Anton Reznicek (University of Michigan), renowned Great Lakes Region botanist, Jim Bissell (Cleveland Museum of Natural Science), and Jim McCormac (Ohio Division of Wildlife) carried special force.

To identify and assess imperilment of plant communities containing ash, we used the U.S. National Vegetation Classification (USNVC) revised in 2008 and subsequently developed by NatureServe Explorer and state Heritage Programs (NatureServe Explorer, 2014). The classification system for the North America's plant community types – a jurisdictional subset of the International Vegetation Classification – is a spatially extensive, range-wide, on-going collaboration of federal, international, academic, and state partners, housed and managed by NatureServe. Our treatment focuses on ecological communities recognized at the Group level in the International Vegetation Classification system and tallies the biological communities in which any one of North America's 16 *Fraxinus* species is named as a dominant or co-dominant plant species. We capitalize Group when the word is meant to convey rank in the USNVC classification.

Ash specialists were defined as those species for which *Fraxinus* was deemed a principal larval or adult host. Stated differently, specialists are those taxa that would be expected to be severely compromised were *Fraxinus* eliminated from the taxon's range. We excluded more than 180 ash-feeding herbivores that are known to feed on hosts outside the family Oleaceae (Gandhi and Herms, 2010; Robinson et al., 2014). Host records were verified by contacting principal taxonomic experts (listed with affiliations in Appendix 2). Frequently, multiple experts, with differing regional data or taxonomic knowledge, were consulted for the same taxon. We were not able to make contact with an active North American thrips (Thysanoptera) systematist and caution that much remains to be learned about North America's mite fauna. Documentation for all taxa and the listed hosts is given in the References column and, as appropriate, in the Comments column of Table 4. Conversations are referenced as "pers. comm." and letters and emails are referenced as "in litt."

We use the term *polyphagous* to refer to cases where herbivores feed on members of more than two families, *oligophagous* to refer to cases where the herbivores feed on more than one genus in the Oleaceae, and *specialist* to refer to cases where *Fraxinus* is the sole or principal host in nature[1]. We restrict use of *monophagous* to those instances where only a single species is (known to be) consumed, and *ecological monophagy* to instances where a species' diet is restricted to a single member of the Oleaceae because only one host species grows in a given geographic location; presumably the herbivore would use other congeners (*Fraxinus*) or perhaps confamilials (Oleaceae) were these available. Throughout this paper, our diet-breadth assessment of invertebrates applies to the most specialized life stage; thus, if a leaf beetle eats just *Fraxinus* as a larva but browses on a spectrum of plants as an adult, our discussion and risk assessment is based on the larval stage.

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[1]This is more restrictive than most insect-plant literature, where oligophagous refers to herbivores that consume plants from just 2-3 families and polyphagous is used to refer to herbivores consuming four or more families.

We follow Hinsinger et al. (2013) in recognizing four Sections of *Fraxinus* in North America. Section is capitalized when the word is meant to convey nomenclatural rank (and phylogenetic membership), i.e., roughly equivalent to a subgenus. Authors for scientific names of imperiled arthropods are given in Table 4; author names for arthropod species not treated in Table 4 are given in the text the first time that taxon is discussed. Arthropod family names are given in the text and Table 4 except where family membership has been made obvious by accompanying text. Authors for *Fraxinus* species appear in the section entitled *North America's Sixteen Fraxinus.*

Species-level taxa (n = 98) were assigned an imperilment or risk category after we had verified host records for each. Four categories of risk were adopted: *Very High*, *High*, *Moderate*, and *Low*, with all examples of the latter excluded from this work. If only *Fraxinus* and native *Chionanthus* L. (fringetree) species were among the reliably reported hosts, individuals were given a *High* risk rating. If additional Oleaceae hosts were recorded, we assigned risk ratings in the follow manner: herbivores known to include *Forestiera* Poir. (swamp privet or desert olive) species were regarded as *Moderate* risk species because native *Forestiera* are abundant enough to serve as an alternative host to *Fraxinus*; ash-feeding and fringetree-feeding

herbivores also reported from non-native Oleaceae hosts, e.g., *Olea* L. (olive), *Ligustrum* L. (privet), and *Syringa* L. (lilac) were given a *High to Moderate* risk rating as these hosts are either considered not abundant enough in wildlands or too infrequently used to serve as viable (sole) hosts for these taxa over extended time periods were ash to be functionally eliminated. If a species was believed to have a strong preference for *Fraxinus* over other Oleaceae, its risk rating was increased; conversely, those with a preference for *Forestiera* resulted in a reduced rating. One species, *Prociphilus americanus* (Aphididae), was determined as *High* risk despite feeding on *Abies* Miller (fir) because both *Fraxinus* and fir are primary hosts in different stages of this aphid's alternating life cycle.

In a few cases where we suspected an apparent specialist may prove to be a polyphage, e.g., *Banasa rolstonii* (Pentatomidae), *Diaspidiotus fraxini*, and *Diaspis fraxini* (both Diaspididae), we invoked the precautionary principle and assigned these species a *High* risk rating. Likewise, species in need of taxonomic study were still included and generally ranked as *High*; e.g., *Sphinx* near *chersis*, *Sympistis fortis*, *Hyrdelia* near *inornata*, and *Zelleria* near *hepariella.* In these cases and others, our thinking is conveyed in the Comments section of Table 4.

Figure 12. Google Earth image of Saint Mary's River west of Decatur, Ohio. The dead trees in this image about the periphery in the riparian corridor east of Rockford are essentially all green ash. The red star indicates the approximate location of the stand shown in Figure 1.

ECOLOGICAL IMPACTS TO NORTH AMERICAN ASH

Flowers et al. (2013) estimate that there are 8.7 billion ash trees and saplings in the lower 48 states – making up roughly 2.5% of the aboveground forest carbon mass in this region. Over most of North America, ash trees tend to grow in mixed hardwood woodlands and forests and are infrequently an ecologically dominant tree at landscape levels. Even in regions of the Midwestern United States, where a significant portion of all *Fraxinus* have been killed, we were unable to easily detect affected communities using Google Earth satellite imagery; although one such example – a severely damaged area along the Saint Mary's River, east of Decatur, Ohio – is shown in Fig. 12. Another important exception are black ash forests, which can be ecologically dominant at larger spatial scales; in many northern woodlands, *F. nigra* grows in virtual monocultures over hundreds of hectares (see below and Figs. 5, 8, 10). Furthermore, over smaller spatial scales, ash species have great ecological importance and, by definition, are essential elements in the woodlands and forests where they are dominant or co-dominant species. The U.S. National Vegetation Classification and NatureServe Explorer (2014) identify 150 U.S. and Canadian forest and shrubland community types where a *Fraxinus* species is named as a dominant or co-dominant element. Just four *Fraxinus* species account for 82% of the 150 community Groups where ash is regarded to be a key community element: green ash (n = 55), white ash (n = 43), black ash (n = 14), and Oregon ash, *Fraxinus latifolia* Benth. (n = 12). Likewise, only eight species of *Fraxinus* grow as the (lead) dominant tree species in 51 community types: green ash (n = 18 community Groups), Oregon ash (n = 10), white ash (n = 8), black ash (n = 6), Carolina ash, *F. caroliniana* Miller (n = 3), blue ash, *F. quadrangulata* Michx. (n = 3), singleleaf ash *F. anomala* (n=2) and pumpkin ash (n = 1). Ecological contributions of each of North America's 16 native *Fraxinus,* as identified in the U.S. National Vegetation Classification and NatureServe Explorer (2014), are summarized in Table 1.

In Table 2 we list 16 North American forest community types where a *Fraxinus* is the dominant tree and the assemblage is regarded as either critically imperiled (G1) or imperiled (G2) in NatureServe Explorer (2014). Most of these imperiled forest types occur south of Pennsylvania; three are restricted to Oregon and Washington. Fortunately, none occur in the most severely affected areas of the Midwestern United States.

A few community types warrant special mention because of their spatial extent or because of their vulnerability. In Wisconsin and elsewhere, swamps with black ash as the sole dominant canopy species may encompass >1000 ha (Fig. 5). Palik et al. (2012) speculated that many such black ash swamps, should ash disappear, may change permanently to shrublands as no other tree species exists in large enough concentrations to form a new canopy. Similarly, along the Atlantic Coastal Plain, Carolina ash sometimes accounts for much of the above-ground biomass in bottomlands and wetlands.

The most significant ecological impacts of EAB to woodlands and forests will be determined by what plant associations establish post-invasion (Flowers et al., 2013; Knight et al., 2013; Burr and McCullough, 2014). Future projections for communities formerly dominated by ash are still a matter of conjecture because no EAB infestations, with their concomitant ecological consequences, have run full course. Undoubtedly, there will be taxonomic variation in which species replace ash due to differences in soil type, hydrology, light, seed banks, and the local pools of potential colonists. In the vicinity of Ann Arbor, Michigan – where EAB was first documented – the ecological vacuum created by the loss of green ash has been filled by spicebush (*Lindera benzoin* L.), pawpaw (*Asimina triloba* Dunal), and prickly ash (*Zanthoxylum americanum* Mill.). In the same area, one wetland formerly dominated by black ash has changed into a monoculture of sedge (*Carex* L.). While both ash habitats changed structurally, neither gave way to invasive species (Anton Reznicek, pers. comm). In Indiana and Ohio, silky (*Cornus amomum* Mill.) and gray dogwoods (*C. racemosa* Lam.) have flourished in communities where ashes (mostly green and black) have been lost (Jim Bissell, Mike Homoya, and Jim McCormac, all pers. comm). Invasive shrubs that have increased

in post-EAB woodlands and bottomlands in Indiana and Ohio include multiflora rose (*Rosa multiflora* Thunb.), honeysuckle (*Lonicera morrowii* A. Gray and three other honeysuckles), and glossy buckthorn (*Rhamnus alnifolia* L'Hér), but not to the extent that many feared. As noted above, even in southeastern Michigan where EAB was first documented, it is still too early to know what the ecological consequences of ash decline will be. Over time, sedge meadows, reed canary grass (*Phalaris arundinacea* L.) bottomlands, and various shrublands that arise following ash die-off will give way to forest communities, but the composition of these replacement forests remains to be seen. It is also too soon to disregard *Fraxinus* as an eventual component of replacement forests as virtually all EAB-affected communities in the Midwestern United States have seedling and sapling ash recruiting presently (Anton Reznicek pers. comm). If native and introduced natural enemies (e.g., Duan et al., 2013; also see Chapters 8 and 9) are able to reduce emerald ash borer density, *Fraxinus* could regain some of its former ecological importance.

Where significant physiognomic changes occur, e.g., where a woodland is initially replaced by a graminoid wetland or shrubland, the biota, species interactions, hydrology (Slesak et al., 2014), light regimen, nutrient cycling, vertebrate food value, and other core ecosystem characteristics will be altered. Beyond the obvious loss of ash-specialized herbivores, structural changes in affected woodlands may change a forest's suitability as breeding habitat and cover for resident vertebrates and invertebrates (e.g., see discussion of mole salamanders [*Ambystoma* species] in pumpkin ash swamps later in this chapter). On a smaller spatial scale, all of the above applies to the forest gaps that form when stands or glades of ash are killed by EAB. Such canopy gaps are noted to cause microclimate effects altering, among other biota, ground beetle populations (Gandhi et al., 2014). These gaps also open up forests for invasions by plant species normally limited by light availability (Herms and McCullough, 2014).

When native plants are replaced by exotic species, such as glossy buckthorn and exotic honeysuckles in Indiana and Ohio (see above), there can be cascading consequences to higher trophic levels. Exotic plants often carry lower herbivore loads (Tallamy and Shropshire, 2009) and as a consequence would not support as many insectivorous birds, mammals, or other wildlife. However, we are compelled to interject here that we generally find ash to be relatively herbivore free. Low insect/pest loading is one reason that ashes are often chosen for city plantings (see species treatments in Elias [1987]). In Tallamy and Shropshire (2009)'s compilation of the Lepidoptera feeding on 1385 plant genera grown in the Mid-Atlantic States, *Fraxinus* ranks sixteenth in richness. Green and white ash trees planted in cities, towns, and parks in the western United States show little evidence of herbivory (DLW pers. observation). Likewise, in European woodlands, ash trees have been documented to have low herbivore loads relative to many other genera of forest trees (Fischbacher et al., 1998). Among four tree genera surveyed near Basel, Switzerland (*Carpinus* L. [hornbeams], *Fagus* L. [beech], *Fraxinus* L. [ash], and *Quercus* L. [oak]), ash yielded only about half the caterpillar frass observed on other surveyed genera. Moreover, while invasive plants generally have depauperate herbivore loads, at least *Lonicera* and *Rhamnus* produce abundant fruits that are exploited by a range of birds and other vertebrates. As with all ecological change, some species will benefit, and others will suffer.

A final note, brought to our attention by Anton Reznicek, is that considerable demographic differences exist among North American *Fraxinus* as to their age and size of first reproduction. Green ash begins flowering and fruiting as a young tree – at diameters frequently ignored by EAB, and thus the species has the potential to persist as young trees, e.g., in open riparian and floodplain communities. By contrast, white and pumpkin ash fruit later, and typically young trees will succumb to EAB infestation before they can bear seed. Hence these species' fate and those of their dependent herbivore faunas are likely to differ substantially from those of green ash.

NORTH AMERICA'S SIXTEEN *FRAXINUS*

Below we provide a synopsis addressing the range, preferred habitat, ecological importance, known susceptibility to EAB, and herbivore specialists of

Table1 . Native ash species found North of Mexico with their USNVC/NatureServe importance and tally of specialist herbivore fauna.

Clade	Species	Common Name	Forest Community Group			Approx. Region	Specialist Herbivores						
			Dominant	Co-dominate	Total		Acari	Coleoptera	Diptera	Hemiptera	Hymenoptera	Lepidoptera	Total Species
Dipetalae	*Fraxinus dipetala*	California Ash		1	1	West	1			1		2	4
	Fraxinus anomala	Singleleaf Ash	2	1	3	Southwest						1	1
	Fraxinus quadrangulata	Blue Ash	3	3	6	East							
Melioides	*Fraxinus cuspidata*	Fragrant Ash				Southwest							
	Fraxinus latifolia	Oregon Ash	10	2	12	West	2	4		4	2	5	17
	Fraxinus papillosa	Chihuahuan ash				South							
	Fraxinus americana	White Ash	8	35	43	Midwest/East	2	11	6	9	3	15	46
	Fraxinus profunda	Pumpkin Ash	1	4	5	East	1						1
	Fraxinus pennsylvanica	Green Ash	18	37	55	Midwest/East	1	6	4	6	1	11	29
	Fraxinus velutina	Velvet Ash		5	5	Southwest	2	5		8		5	20
	Fraxinus berlandieriana	Mexican Ash		2	2	Texas		3					3
	Fraxinus caroliniana	Carolina Ash	3	6	9	Southeast						1	1
	Fraxinus albicans	Texas Ash		2	2	Texas	1	1					2
Pauciflorae	*Fraxinus greggii*	Gregg's Ash				Texas					1	2	3
	Fraxinus gooddingii	Goodding's Ash				Arizona						1	1
Fraxinus	*Fraxinus nigra*	Black Ash	6	8	14	Midwest/Northeast	1	1		1		7	10
NA	*Fraxinus spp.*	various ashes	1	1	1	East	1	1	1	5		2	10

Footnote: In-the-wild host ranges are poorly known for most of the arthropod species in this table. We suspect that virtually all of the taxa that appear to be specialized in this table, in fact, have broader host ranges than indicated above due to undersampling—see *Feeding Guilds and Specificity of Arthropod Herbivores* discussion. Diets of southern and western taxa are especially poorly known.

Table 2. Sixteen globally imperiled forest communities dominated by *Fraxinus*. See NatureServe Explorer (2014) for details on distribution, ranking, and additional plant associations.

Ecological Community dominated by *Fraxinus sp.*	NatureServe Status	Distribution*	Division
Fraxinus americana / Andropogon gerardii - Sorghastrum nutans - Schizachyrium scoparium - Pycnanthemum tenuifolium Herbaceous Vegetation	Critically imperiled	MD, VA	Eastern North American Dune & Coastal Grassland & Shrubland
Fraxinus americana - Juniperus virginiana / Talinum teretifolium - Polygonum tenue - Opuntia humifusa Wooded Herbaceous Vegetation	Critically imperiled	VA	Eastern North American Grassland, Meadow & Shrubland
Fraxinus americana / Physocarpus opulifolius / Carex pensylvanica - Allium cernuum - (Phacelia dubia) Wooded Herbaceous Vegetation	Imperiled	VA	Eastern North American Grassland, Meadow & Shrubland
Fraxinus americana - Carya glabra / Symphoricarpos orbiculatus - Rhus aromatica / Piptochaetium avenaceum Woodland	Imperiled	GA, NC	Eastern North American Cool Temperate Forest
Fraxinus americana - Carya ovata / Frangula caroliniana / Helianthus hirsutus Woodland	Critically imperiled?	VA	Eastern North American Cool Temperate Forest
Fraxinus americana - Carya glabra / Muhlenbergia sobolifera - Helianthus divaricatus - Solidago ulmifolia Woodland	Imperiled	MD, VA, WV	Eastern North American Cool Temperate Forest
Fraxinus americana - Juglans nigra - Ulmus rubra / Acer barbatum - Ostrya virginiana / Ptelea trifoliata Forest	Imperiled	AL?, FL, GA?	Southeastern North American Warm Temperate Forest
Fraxinus latifolia / Carex deweyana - Urtica dioica Forest	Critically imperiled	OR, WA	Vancouverian Flooded & Swamp Forest
Fraxinus latifolia / Juncus patens Forest	Imperiled	OR	Vancouverian Flooded & Swamp Forest
Fraxinus latifolia - Populus balsamifera ssp. trichocarpa / Rubus spectabilis Forest	Imperiled	OR, WA?	Vancouverian Flooded & Swamp Forest
Fraxinus nigra - Abies balsamea / Rhamnus alnifolia Forest	Critically imperiled	PA?, WV	Eastern North American Flooded & Swamp Forest
Fraxinus pennsylvanica - (Ulmus americana) - Pinus taeda / Morella cerifera - Juniperus virginiana var. silicicola Tidal Forest	Critically imperiled - Imperiled	NC	Eastern North American Flooded & Swamp Forest
Fraxinus pennsylvanica - (Carya aquatica) / Forestiera acuminata / Phanopyrum gymnocarpon Depression Forest	Imperiled?	TX	Southeastern North American Flooded & Swamp Forest
Fraxinus pennsylvanica - Populus heterophylla - Ulmus americana - (Quercus texana) Forest	Imperiled?	AR?, LA	Southeastern North American Flooded & Swamp Forest
Fraxinus quadrangulata - Juniperus virginiana var. virginiana / Schizachyrium scoparium - Lithospermum canescens Woodland	Imperiled	KY	Eastern North American Cool Temperate Forest
Fraxinus quadrangulata - Quercus macrocarpa - Quercus muehlenbergii / Arundinaria gigantea ssp. gigantea / Elymus spp. Woodland	Critically imperiled?	KY	Eastern North American Cool Temperate Forest

* AL - Alabama, AR - Arkansas; FL - Florida; GA - Georgia; KY - Kentucky; LA - Louisiana; MD - Maryland; NC - North Carolina; OR - Oregon; PA - Pennsylvania; TX - Texas; VA - Virginia; WA - Washington; WV - West Virginia

each North American *Fraxinus*. We group ash species by four phylogenetic sections based on Hinsinger et al. (2013), beginning with the nominate Section *Fraxinus*, which is monotypic in the Americas and represented only by black ash (*F. nigra*). Of these four phylogenetic groupings, Section Melioides (in its broad sense) contains the most ecologically important, widespread species of ash; it is also the most speciose section, with 10 North American species. For the purposes of our evaluation, we make the assumption that all 16 North American species are vulnerable to the emerald ash borer, given initial host preference studies and the uncertainty in climate niche models for the insect (Sobek-Swant et al., 2012; Liang and Fei, 2014). However, it seems unlikely to us that the beetle will pose a major threat to the nine species of western arid land ash (*F. albicans* Buckley*, F. anomala* Torr. ex S. Watson*, F. berlandieriana* DC.*, F. cuspidata* Torr.*, F. dipetala* Hook. & Arn.*, F. gooddingii* Little*, F. greggii, F. papillosa* Lingelsh, and *F. velutina* Torr.), given that EAB does not inhabit xeric and desert areas in its native range.

Section *Fraxinus*

Black ash. *Fraxinus nigra*, a small tree that is the most northern member of its genus, is distributed across southern Canada from eastern Manitoba to Newfoundland, south in mountains through West Virginia, in the Ohio River Valley to southern Indiana and Illinois, and in much of Iowa (Elias, 1987). Black ash is a major component of 14 forest community Groups (NatureServe Explorer, 2014): six as a dominant tree species and eight as a co-dominate. It is the dominant tree in one critically imperiled (G1) forest community type, the *Fraxinus nigra - Abies balsamea /Rhamnus alnifolia* forests of West Virginia and Pennsylvania – two states with established EAB populations.

Black ash grows in damp woods, bottomlands, swamps, and other wetlands; it tolerates considerable inundation (Fig. 8). Common associates include black spruce (*Picea mariana* [Mill.] Britton, Sterns & Poggenb.), white cedar (*Thuja occidentalis* L.), tamarack (*Larix laricina* [Du Roi] K. Koch), birch (*Betula* L.), and especially red maple (*Acer rubrum* L.). It sometimes grows in nearly pure stands over

hundreds of hectares, principally in the North American Great Lakes region (Fig. 5). Red maple-black ash swamps cover thousands of hectares in southern Canada and the northern United States. Where *Fraxinus nigra* grows in monocultures or as a dominant in either the canopy or subcanopy, the emerald ash borer represents a special threat. Black ash is considered to be the most vulnerable North American ash species as its range lies within the presumed climate niche for the beetle (Klooster et al., 2014; Liang and Fei, 2014; Leah Bauer pers. comm.). Of additional concern is how plant community composition will change in black ash stands post-EAB invasion. Where the canopies are lost there is added risk that the local biota, forest structure, hydrology, and other core ecological attributes will be harmed.

We recorded ten ash specialist herbivores from black ash: one aphid, one seed weevil, one sawfly, one gracillariid leafminer, two noctuids, one pyralid, and three sphingids (Table 4). All but one of these arthropods are also known from Section Melioides sensu stricto *Fraxinus*, and especially *F. americana*, *F. pennsylvanica*, or both. The rarely encountered Canadian sphinx (*Sphinx canadensis*) (Sphingidae) warrants special consideration. Tuttle (2007) wrote: "In northeastern Indiana, [*Sphinx canadensis*] is closely associated with the understory of dense hardwood stands along the perimeter of wetlands. Over several seasons larvae were found exclusively on black ash (*Fraxinus nigra*: Oleaeceae), although two additional ash species [*F. americana*, *F. pennsylvanica*] were present. Just as significant, larvae were never found on black ashes growing in open areas, although those same trees supported the larvae of two other sphingid species." The northern range of the Canadian sphinx closely follows that of black ash. Southward the moth's range extends beyond that of *F. nigra,* south of the Ohio River, where the species is rare and its *Fraxinus* hosts are unknown.

Section Pauciflorae

Goodding's ash. *Fraxinus gooddingii* is a narrowly distributed shrubby ash limited to southeastern Arizona and northern Sonora. It grows on rocky slopes (often on limestone), in desert scrub, oak woodlands, and riparian associations, scattered

among other woody shrubs on lower canyon slopes; most records are from arroyos and canyon bottoms from 1100 to 1500 meters (SEINet, 2014).

No previous literature has identified specialist herbivores on this host. Jim Verrier and DLW found *Philtraea elegantaria* (Geometridae) feeding on Goodding's ash in Rock Corral Canyon in the Tumacácori Range. *Sphinx libocedrus* (Sphingidae) feeds on this ash in Arizona (Tuttle, 2007), but species of *Forestiera* are presumed to be its principal hosts.

Gregg's ash. *Fraxinus greggii* is found in the Trans-Pecos region of southwestern Texas south at least to Hidalgo, Mexico. Gregg's ash is often limited to riparian corridors in arroyos, canyon bottoms, and along water courses from 400 to 1800 meters (Powell, 1998; SEINet, 2014). It grows on cliffs, rocky slopes, and canyon bottoms, frequently on limestone. Throughout its range, it grows as a subdominant: typically as scattered plants, in desert canyons and foothills, usually upslope from water.

Noel McFarland (pers. comm.) found caterpillars of *Philtraea paucimacula* (Geometridae) in high densities on Gregg's ash near Laredo, Texas. *Sphinx libocedrus* (Sphingidae) is known from this ash, as well as *Forestiera* (Table 4).

Section Melioides

White ash. *Fraxinus americana* is the second most abundant and widespread native North American ash. It is a common component in many forest types, including bottomlands, open upslope woodlands (Fig. 11), and mixed hardwood forests. Typically, white ash occurs in sites with enriched, but well drained, moist soils, where it grows in association with sugar maple (*Acer saccharum* Marshall), tulip tree (*Liriodendron tulipifera* L.), cherry (*Prunus* L.), beech, sweet gum (*Liquidambar styraciflua* L.), red maple, and willow (*Salix* L.). NatureServe Explorer (2014) identifies eight ecosystems with white ash as a dominant tree and 35 where it is a co-dominant. Of the eight forest community Groups where white ash is dominant, three are critically imperiled (G1) and four are imperiled (G2) (NatureServe Explorer, 2014) (Table 2). White ash seeds are eaten by many birds and squirrels (Burns and Honkala, 1990) and it is

commonly planted as shade trees in yards, parks, and along streets (Elias, 1987; Burns and Honkala, 1990). EAB is thought to prefer other *Fraxinus* species over white ash (Anulewicz et al., 2008), but *F. americana* is still attacked and generally suffers very high mortality. However, apparent resistance of white ash to EAB has recently been seen in Ohio, with some white ash trees surviving in forests where green ash have succumbed to the beetle (Jim Bissell pers. comm.).

White ash has the richest fauna of ash-specialized herbivores in North America – three times as many as any western ash. We list 46 species in Table 4, but suspect that virtually all of the eastern specialists listed only from *Fraxinus* (without an associated species epithet) probably use white ash. Specialist arthropods feeding on *F. americana* include 2 mites, 1 leaf beetle, 5 bark beetles, 4 seed weevils, 1 scarabaeid, 1 agromyzid, 5 gall midges, 2 aphids, 6 mirids, 1 lace bug, 3 sawflies, 2 inchworms, 2 gracillariid miners, 3 owlets (Noctuidae), 1 pyralid, 1 clearwing borer, and 5 sphingids (Table 4). Sixteen herbivores are recorded only from white ash: 2 *Hylesinus* (Curculionidae), 2 *Lignyodes* (Curculionidae), 1 *Xyloryctes* (Scarabaeidae), 4 *Dasineura* (Cecidomyiidae), 1 *Prociphilus* (Aphididae), 3 *Tropidosteptes* (Miridae), 1 *Tethida* (Tenthredinidae), 1 *Hydrelia* (Geometridae), and 1 *Copivaleria* (Noctuidae), although none of these are known to be strictly monophagous.

Green ash. *Fraxinus pennsylvanica* is a small-to-medium-sized, fast growing tree that is the most widely distributed ash species in North America, extending from southwestern Saskatchewan to Cape Breton Island, south to northern Florida and eastern Texas. It is an abundant, ecologically important species throughout much of this range and thrives in floodplains and other bottomlands. Elias (1987) notes that *F. pennsylvanica* is especially abundant through the Mississippi Valley. Green ash is the most common *Fraxinus* to grow as a dominant tree in the U.S. National Vegetation Classification (USNVC) system. Fifty-five ecological community Groups contain *F. pennsylvanica* as a dominant (n = 18) or co-dominant tree (n = 37) (NatureServe Explorer, 2014). Green ash is the dominant tree in three forest Groups considered to be Critically Imperiled to Imperiled; all three of which are flooded swamp forests in the

southeastern United States (Table 2). In forests where green ash is dominant, Burr and McCullough (2014) note that green ash is "unlikely to persist as a dominant species" after EAB invasion. Its seeds are an important forage for a variety of birds, small mammals, and other wildlife; deer and moose (*Alces alces L.*) feed on new growth (Elias, 1987). Its strong wood is sold commercially for bats, oars, and tool handles but is not as desirable for wood working as white ash (Burns and Honkala, 1990).

We record 29 *Fraxinus*-Oleaceae specialists from green ash; thus, it ranks only behind white ash in its importance to *Fraxinus*-specialist herbivores: 1 mite, 1 buprestid, 3 bark beetles (*Hylesinus*), 2 seed weevils (*Lignyodes*), 1 agromyzid, 3 gall midges (Cecidomyiidae), 1 aphid, 5 mirids, 1 sawfly, 4 gracillariid miners, 2 owlets (Noctuidae), 1 pyralid, 1 clearwing borer (Sesiidae), and 3 sphingids. Seven species are recorded only from green ash: one undescribed *Dasineura* (Cecidomyiidae), two *Tropidosteptes* (Miridae), and the two gracillariids *Marmara basidendroca* and *Marmara corticola*. The last two of these, so far as known, are monophages, at least at the type locality and surrounding areas of upstate New York (Fitzgerald, 1973; Terry Fitzgerald pers. comm.). See also *Imperilment Risk Rating* discussion.

Mexican ash. *Fraxinus berlandieriana* is a small tree that occurs from the vicinity of Austin, Texas southward and westward into Mexico. The core of its range is in the Mexican states of Coahuila, Nuevo Leon, and Tamaulipas. It grows in moist canyons and along streams and rivers of the Rio Grande Plains and southern prairies (Vines, 1984) and is frequently planted as a shade tree in parks and cities. Two community types in Texas include Mexican ash as a co-dominate (n = 2), one of which is considered critically imperiled/imperiled (G1G2) (NatureServe Explorer, 2014).

Ash specialists reported from the United States include the buprestid *Trigonogya reticulaticollis*, the chrysomelid *Capraita sexmaculata*, and the seed weevil *Lignyodes helvolus*. While Mexican ash is the only reported host for *T. reticulaticollis*, based on the biology of related metallic wood boring beetles, it is probable that its host range will be found to include other Oleaceae.

Carolina ash. *Fraxinus caroliniana* is a small tree of the Atlantic Coast Plain from Virginia, south through much of Florida, west to east Texas and southern Arkansas. It grows in swamps, wetlands, bottomlands, and other mesic to wet forest types. Like many other members of the genus, it does especially well in marl soils (SEINet, 2014). Carolina ash commonly grows with gums (*Nyssa* L.), hollies (*Ilex* L.), sweet gum, sugarberry (*Celtis laevigata* Willdenow), and others (Elias, 1987); it can be locally abundant as an understory member of bald cypress-tupelo swamps (SEINet, 2014). *Fraxinus caroliniana* is a dominant in nine forest community Groups: three as a dominant and six as a co-dominant tree (NatureServe Explorer, 2014). It occurs in dense monospecific stands in coastal plain areas of Louisiana and Texas (in "*Fraxinus caroliniana* Seasonally Flooded Forests"), which are deemed G2G3 imperiled-vulnerable communities. Only one herbivore, the sphingid moth *Ceratomia undulosa*, an oligophage on Oleaceae, is recorded from this *Fraxinus*. The lack of feeding records for this ash is almost certainly an artifact of limited sampling, presumably because its preferred habitat is in swamps and wetlands and it is not a commercially important ash.

Fragrant ash. *Fraxinus cuspidata* is a shrub (or sometimes small tree) that is found in scattered populations across the southwestern United States from northwestern Arizona to western Texas and south into Mexico, mostly in the foothills of desert ranges (SEINet, 2014). It prefers well-drained, rocky soils in canyons, and north facing cliffs of limestone, sandstone, or igneous soils (Elias, 1987; Powell, 1998; SEINet, 2014). It tends to occur in low densities intermixed among oaks, leguminous trees, and other woody plants.

Two specialist herbivores have been recorded from this small ash: DLW collected caterpillars of a *Sympistis heterogena* (Noctuidae) feeding on new spring leaves in May 2014 and a second unidentified noctuid[2] species in July of 2014.

Oregon ash. *Fraxinus latifolia* is a Pacific Coast tree that grows from the Olympia area in

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[2] The caterpillar, seen in late July, was banded with smoky red in the early instars and is a new foliage specialist.

western Washington south to the San Francisco Bay Area (California) and southern Sierra Nevada, as well as in disjunct populations in southern California's mountains. The tree grows in "moist rich soils along streams and rivers, and in canyons" to 500 meters (Elias, 1987). It is an abundant and ecologically important tree along riparian corridors throughout much of its range, and is the only commercially important *Fraxinus* in western North America. Oregon ash is a dominant tree in ten forest community Groups and a co-dominant in two others. While it will grow in monocultures, at least along streams and in floodplains, it more typically grows in mixed hardwood stands with maple, alder (*Alnus* Mill.), poplar (*Populus* L.), and willow; Elias (1987) also adds California laurel (*Umbellularia californica* [Hook. & Arn.] Nutt.) and grand fir (*Abies grandis* [Douglas ex D. Don] Lindley) as associates of *F. latifolia*. Three forest communities in the Pacific Northwest dominated by Oregon ash are regarded as critically imperiled/imperiled in NatureServe Explorer (2014) (See Table 2).

We record 17 species of ash specialists from Oregon ash, many for the first time: 2 mites, 2 bark beetles, 2 seed weevils, 1 aphid, 2 mirids, 1 tingid, 2 sawflies, and 1 inchworm (Geometridae), 1 gracillariid leafminer, 1 owlet moth, and 1 ermine moth (Yponomeutidae). Five of these are only known from Oregon ash: *Hylesinus oregonus* (Curculionidae: Scolytinae); *Lignyodes auratus* (Curculionidae: Curculioninae); *Philtraea latifoliae* (Geometridae), *Caloptilia* n. sp. (Gracillariidae), and *Sympistis fortis* (Noctuidae). We suspect that their strict monophagy is either an artifact of undersampling or because *Fraxinus* specialists whose ranges are restricted to the Sierra and areas north of San Francisco are monophagous simply because *F. latifolia* is the only ash that grows where they occur.

Chihuahua ash. *Fraxinus papillosa* is a geographically restricted ash that grows to become a small tree. It is found in small populations through desert ranges of southeast Arizona, southwestern New Mexico, the Chinati Mountains of west Texas, and southward into the Sierra Madre Occidental Mountains of Mexico (Elias, 1987; Powell, 1998). Typically *F. papillosa* grows in canyon bottoms and

on north-facing cliffs (SEINet, 2014). Like other desert ashes, it occurs as scattered plants in woody associations near water or in microhabitats with reduced moisture stress. No ash specialists are currently recorded on *F. papillosa*. In Hinsinger et al.'s (2013) study, Chihuahuan ash grouped with Oregon ash (*F. latifolia*), in a clade outside of all other members of the Melioides sensu stricto section of the genus. Because of this phylogenetic difference and its taxonomic and geographic isolation, Chihuahuan ash strikes us as a likely candidate for hosting unique herbivores.

Pumpkin ash. *Fraxinus profunda* grows in scattered locations across eastern North America, with most occurrences along the Atlantic Coastal Plain and through the Ohio and Mississippi River Valleys. It is restricted to very wet soils that include bottomlands, floodplains, swamps (especially bald cypress and tupelo swamps), and coastal marshes (Elias, 1987; Burns and Honkala, 1990; Nesom, 2010) (Figs. 7, 9). *Fraxinus profunda* is a dominant (n = 1) and co-dominant (n = 4) in five eastern forest community types, one of which is imperiled (NatureServe Explorer, 2014). This ash species is quite susceptible to EAB; devastated would not be an overstated descriptor for some local Ohio preserves where pumpkin ash once grew as a dominant species (Jim Bissell pers. comm.)

Only one arthropod species is recorded from *F. profunda*: a mite that is not a specialist on pumpkin ash (Table 1). No doubt pumpkin ash's poorly sampled herbivore fauna is a reflection of the difficulty involved with sampling the inundated wetlands where this tree thrives. Certainly it is a candidate for more study, especially given its susceptibility and because its range falls entirely within the predicted climatic niche of EAB.

Velvet ash. *Fraxinus velutina* is a small ash of the southwestern United States and northwestern Mexico. Its U. S. range is concentrated in Arizona and extends westward through arid portions of southern California, Nevada, Utah, and eastward into the Trans-Pecos region of Texas. It grows near streams, rivers, washes, and other areas of reduced moisture stress. Common associates include hackberry (*Celtis occidentalis* L.), oak, poplar, sycamore (*Platanus* L.), and willow. It reaches greatest importance in mesic stretches of

canyons between 1200 and 1600 meters where it "can form almost pure stands" (Elias, 1987). Velvet ash is listed as a co-dominant tree in five forest community Groups in NatureServe Explorer (2014).

We record 20 ash specialist herbivores from velvet ash, the third most of any *Fraxinus* species and the greatest number for any western species: 2 mites, 1 bark beetle, 2 seed weevils, 2 scarab beetles, 2 aphids, 2 scale insects, 2 plant bugs, 1 stink big, 1 lace bug, 2 owlet moths (Noctuidae), and 3 sphingids. Most of these are known only from *F. velutina*: the mite *Brevipalpus cardinalis* (Tenuipalpidae), the weevil *Lignyodes arizonicus* (Curculionidae), the beetles *Dynastes granti* and *Xyloryctes thestalus* (both Scarabaeidae), the armored scales *Diaspidiotus fraxini* and *Diaspis fraxini* (both Diaspididae), the plant bug *Tropidosteptes illitus* (Miridae), the stink bug *B. rolstoni* (Pentatomidae), and the moths *Chloronycta tybo* and *Sympistis punctilinea* (both Noctuidae) and *Ceratomia sonorensis* and *Sphinx* near *chersis* (both Sphingidae). In the Sonoran and Chihuahuan deserts of the western United States, velvet ash is the only *Fraxinus* considered to be an ecologically dominant or, more commonly, co-dominant species. Consequently, the loss of velvet ash would have an impact on all southwestern *Fraxinus* herbivores, given that it accounts for most of the ash biomass in the southwestern United States.

Section Dipetalae

Singleleaf ash. *Fraxinus anomala* is a shrub or small spreading tree that grows in canyons, desert drainages, washes, and along water bodies of the North American Great Basin region. It exists as scattered stands in western Colorado, Utah (where it is widespread), southern Nevada, and southeastern California, central and northern Arizona, and extreme northwestern New Mexico (SEINet, 2014). *Fraxinus anomala* is a common riparian element between 600-1,900 meters. NatureServe Explorer (2014) recognizes singleleaf ash as a dominant (n = 2) or co-dominant (n = 1) in three southwestern plant community associations (all three are currently unranked or unrankable due to lack of data). We do not know of any specialist ash herbivores from *F. anomala*, although we suspect that it is the primary

host for *Philatraea utahensis* Buckett.

California or two-petal ash. *Fraxinus dipetala* is a shrubby ash that grows in scattered populations in central and southern California's coastal ranges and the foothills of the Sierra Nevada Mountains. It thrives either on slightly acidic granite slopes, often near watercourses, or on slightly alkaline clays in chaparral associations. Below 500 meters, it is usually restricted to the upslope vicinities of washes and watercourses. Above this, California ash occurs in chaparral and other foothill communities, typically as a scattered element growing with *Pinus* L. (pine), oak, *Arctostaphylos* Adans. (manzanita), and others (Elias, 1987; SEINet, 2014). *Fraxinus dipetala* is listed as a co-dominant (along with *Prunus ilicifolia* [Nutt. ex Hook. & Arn.] Walp.) in one U.S. forest community Group by NatureServe Explorer (2014).

The herbivore fauna of California ash is not well known – the plant is easily overlooked, and rarely common. Four ash-specialist herbivores are recorded from California ash: 1 mite, 1 aphid, 1 inchworm (Geometridae), and 1 ermine moth (Yponomeutidae). Two moths are known exclusively from this ash: *Philtraea surcaliforniae* (Geometridae) and *Zelleria* near *semitincta* (Yponomeutidae) (Buckett, 1970) (Table 4).

Blue ash. *Fraxinus quadrangulata* is a small to large tree (Fig. 6) whose core distribution is in the Midwestern United States from eastern Kansas to extreme southern Ontario, south into Arkansas and northern Alabama. Blue ash grows in upland woods and on slopes usually over limestone, but also in moist woodlands and evidently even bottomland forests (Elias, 1987). Typically it occurs as a subdominant species (n = 3) intermixed in stands dominated by oak, mockernut hickory (*Carya tomentosa* Sarg.), sweet gum, and others. It is listed as the dominant tree species in three Kentucky forest community types (NatureServe Explorer, 2014): critically imperiled (G1) blue ash-oak savannah woodlands (Bryant et al., 1980; NatureServe Explorer, 2014); imperiled (G2) blue ash - eastern red-cedar / little bluestem - hoary puccoon woodlands; and possibly no longer extant (GH) *Fraxinus quadrangulata - Quercus macrocarpa / Arundinaria gigantea* ssp. *gigantea* wooded shrublands. *Fraxinus*

quadrangulata is believed to be the most EAB-resistant *Fraxinus* in eastern North America and, consequently, its relative abundance may increase in woodlands and forests where other ash succumb to the beetle (Anulewicz et al., 2007; Tanis and McCullough, 2012).

Blue ash has a surprisingly modest herbivore fauna. Only one ash specialist is recorded from this ash, the moth *Plagodis kuetzingi* (Geometridae), which also occurs on *F. americana* and presumably other ashes. Robinson et al. (2014) lists only two generalist Lepidoptera for blue ash. Its diminished herbivore fauna no doubt is a reflection of its taxonomic isolation: it is the only member of the Dipetalae Section in eastern North America (Hinsinger et al., 2013). Given the above, perhaps it is not surprising that blue ash is more resistant to EAB than other eastern members of the genus. Given that it represents a unique taxonomic entity in eastern North America, blue ash is a candidate for a focused herbivore survey, especially for cecidomyiids, leaf or bast miners, and other herbivores known to show extreme monophagy.

Texas ash. *Fraxinus albicans* (= *F. texensis*) is a small drought-tolerant relative of white ash that grows on limestone from the Arbuckle Mountains of southern Oklahoma southward across the Edwards Plateau into south central Texas (Vines, 1984; Elias, 1987). It is a co-dominant (with oak) in two forest community types (NatureServe Explorer, 2014). We know of only two reports of specialist herbivores from Texas ash: 1 mite and 1 weevil, neither of which is monophagous on *F. albicans*. Robinson et al. (2014) does not list any lepidopterans from Texas ash. The dearth of feeding records for this ash is surely a reflection of its limited geographic distribution and thus a sampling artifact. Presumably because of its close relation to *F. americana* (Vines, 1984), *F. caroliniana*, and other members of the Melioides *sensu stricto* section of the genus (Hinsinger et al., 2013), *F. albicans* will be found to share many herbivores with other *Fraxinus*.

EFFECTS ON VERTEBRATES

Martin et al. (1951) regarded North American ashes to be "only of moderate importance to wildlife." No vertebrates are monophagous or otherwise exclusively dependent on ash or ash-dominated communities, although some local populations might be energetically or ecologically dependent on ash simply due to the fact that *Fraxinus* are the dominant or co-dominant trees in the woodland or forest types where they live (northern black ash swamps provide compelling examples of such) (Fig. 5). A summary of vertebrates using ash as a food source is given in Table 3, with the proviso that the published literature is greatly biased towards game species. We also note that the literature for vertebrates is largely qualitative, with little data on the importance of ash relative to other food resources; we suspect that the paucity of studies addressing the ecological importance of ash may be a reflection of the fact that, by itself, ash trees rarely serve as a core food or sheltering resource.

We know most about the six species of *Fraxinus* where ash has been identified as being a community dominant tree species: black, blue, green, Oregon, pumpkin, and white. Among mammals, ungulates and lagomorphs (rabbits) are often mentioned as feeding on non-woody tissues of ash. Northward, green and especially black ash are browse sources for moose (Elias, 1987) and the white-tailed deer (*Odocoileus virginianus* Zimmermann). A wide range of rodents, but especially chipmunks, mice, and squirrels, consume the winged seeds. And, while we found little mention of such in the literature, undoubtedly North American beavers (*Castor canadensis* Kuhl) consume ash across their range. Ash seeds are regarded to be of moderate importance to seed-eating, woodland or forest dwelling birds such as cardinals, chickadees, finches, and grosbeaks. Grouse, quail, and turkey are ground feeders known to consume ash seeds (Martin et al., 1951). Given the propensity of the genus for wetlands, the importance of ash seeds to waterfowl has likely been underestimated – we only found specific mention of wood ducks (*Aix sponsa* [L.]) feeding on ash samaras (Martin et al., 1951). In forests where *Fraxinus* are dominants, ashes also provide cover (e.g., for

Table 3. Vertebrates that feed on *Fraxinus*.

Ash Tree	Mammals	Birds	Literature	Comments
Fraxinus albicans				
Fraxinus americana	white tail deer, fox squirrels	wood ducks, quail, turkey, grouse, bobwhite, finches (esp. purple finch), grosbeaks (esp. pine grosbeak), cardinals, other songbirds	Elias, 1987; Burns and Honkala, 1990	
Fraxinus anomala				
Fraxinus berlandieriana				
Fraxinus caroliniana				
Fraxinus cuspidata	deer			
Fraxinus dipetala				
Fraxinus gooddingii				
Fraxinus greggii	deer, rabbits, ground squirrels, other rodents	many birds		
Fraxinus nigra	white tail deer, moose, small mammals	wood ducks, grouse, turkey, song birds	Elias, 1987; Burns and Honkala, 1990	
Fraxinus papillosa				
Fraxinus profunda	white tail deer	wood ducks, many other birds	Elias, 1987; Burns and Honkala, 1990	
Fraxinus pennsylvanica	white tail deer, mule deer, moose, rabbits, squirrels, and other rodents	wood ducks, quail, turkey, cardinals, finches	Elias, 1987; Burns and Honkala, 1990	large seed crops
Fraxinus quadrangulata	white tail deer	wood ducks, quail, turkey, songbirds	Elias, 1987	
Fraxinus nigra	white tail deer, moose, small mammals	wood ducks, grouse, turkey, song birds	Elias, 1987; Burns and Honkala, 1990	
Fraxinus velutina			Elias, 1987	"little value to wildlife"
Fraxinus spp.	chipmunks		Martin et al., 1951; Rhoads and Block, 2005	
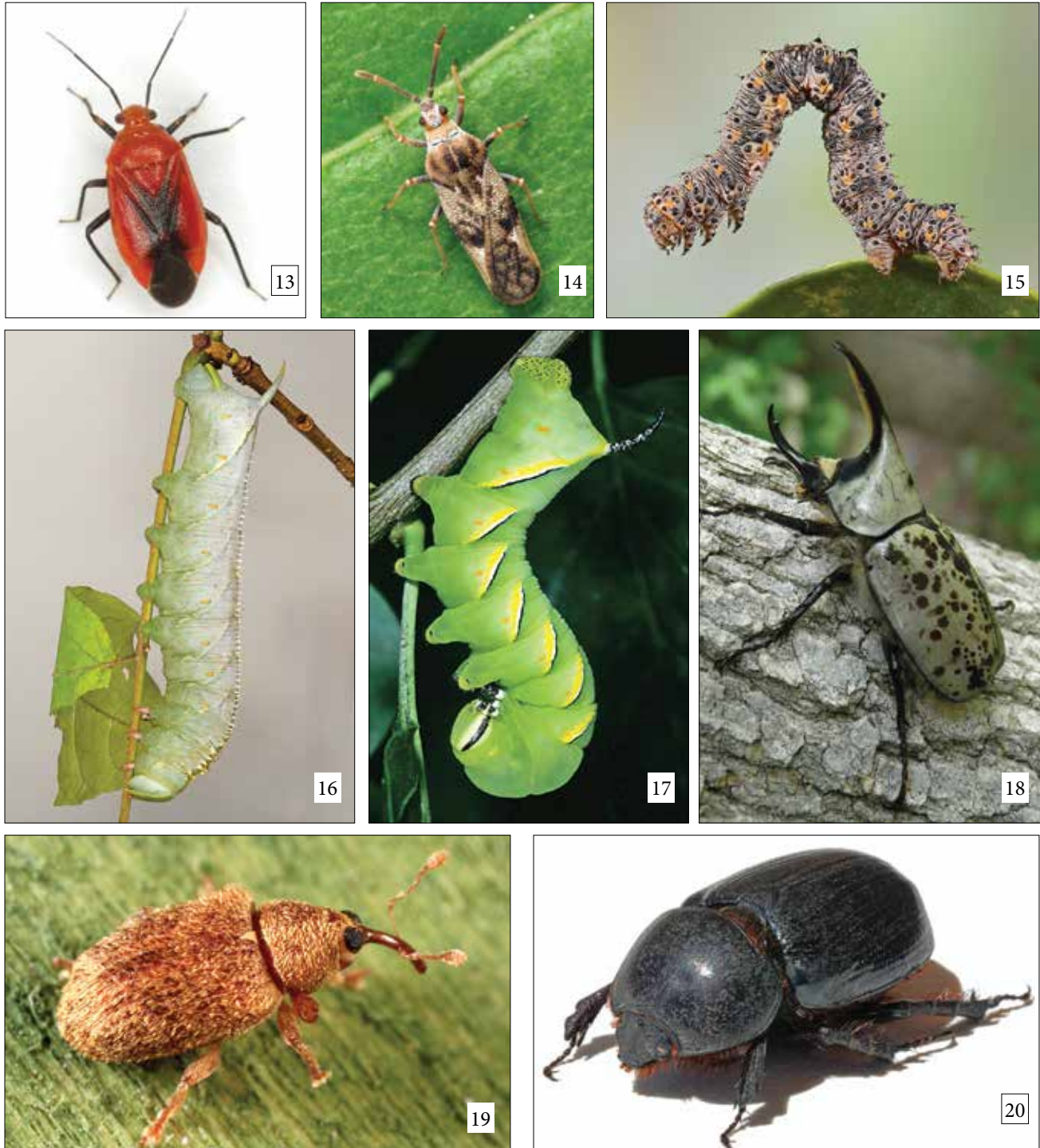
Figures 13 - 20. Ash-specialist insects that represent some of the larger taxa threatened by EAB. (13) *Tropidosteptes cardinalis*, Family Miridae (Photo courtesy Mike Quinn); (14) *Leptoypha mutica*, Family Tingidae. (Photo courtesy Tom Murray); (15) *Philtraea elegantaria*, Family Geometridae (Photo DLW); (16) *Sphinx franckii*, Family Sphingidae (Photo DLW); (17) *Sphinx kalmiae*, Family Sphingidae (Photo DLW); (18) *Dynastes granti*, Family Scarabaeidae (Photo courtesy Margarethe Brummerman); (19) *Lignyodes helvolus*, Family Curculionidae (Photo courtesy Bill Johnson); (20) *Xylorctes thestalus*, Family Scrarabeidae. (Photo courtesy Richard Hayes)

moose [Gould and Bauer, 2009]) and above-ground structure important to vertebrate wildlife for roosting and nesting. Ash trees with broken tops or otherwise damaged stems sometimes form cavities which are used by a variety of birds, bats, and other animals.

One ash community type warrants special mention, i.e., woodland pools dominated by pumpkin ash (*F. profunda*) (Figs. 7, 9). In southeastern Michigan, portions of Ohio, and presumably elsewhere, pumpkin ash is sometimes the sole or dominant tree that grows in the standing water of vernal and permanent (fishless) forest pools and as such provides all or much of the canopy closure. Ash-dominated woodland and forest pools are an important breeding habitat for five species of mole salamanders in the Midwestern United States (spotted [*Ambystoma maculatum* Shaw], Jefferson [*A. jeffersonianum* (Green)], blue-spotted [*A. laterale* Hallowell], small-mouthed [*A. texanum* Matthes], and marbled [*A. opacum* Gravenhorst]), and an occasional breeding habitat for the eastern tiger salamander (*A. tigrinum* Green). Many of these salamanders are important conservation targets that receive legal protection. Of these, the marbled salamander may be the most vulnerable because females lay their eggs in mud during the fall, in anticipation of autumn and winter rains (Degraaf and Rudis, 1986; Pfingsten et al., 2013; Kenney, 1995). Greater sun exposure due to canopy loss could affect the hydrology of wetlands, especially in the fall, before rains and snow melt have had a chance to fill them. Most worrisome is that, in Ohio, pumpkin ash is sometimes the only tree found growing in these saturated to flooded soils where mole salamanders and other wildlife breed (Jim McCormac, pers. comm.).

While much wildlife will experience detrimental effects from ash elimination, no doubt some vertebrates will benefit. Where EAB has invaded in the Midwestern United States, woodpecker numbers have spiked in the vicinity of infestations because EAB larvae serve as an abundant, easily harvested food resource and dying ash provide nesting trees for the birds (Cappaert et al., 2005b; Koenig et al., 2013). Similarly, standing dead ash trees are preferred roosting and nesting sites for many other vertebrates and will, for a limited time, benefit wildlife posthumously. Canopy gaps left by these dying ash

species may also allow fruit-producing shrubs such as *Cornus*, *Lonicera,* and *Rhamnus* (two of which are, unfortunately, non-native invasives) to colonize and thrive, the fruits of which will benefit a variety of fruit-eating birds and mammals.

EFFECTS ON ASH-FEEDING INVERTEBRATES

We identify 98 *Fraxinus*-dependent invertebrate herbivores (or inquilines) as potentially threatened by the spread of EAB, 45 of which are reported here for the first time (Figs. 13-20). Because our compilation of *Fraxinus* feeders was a bottom-up tabulation for all insects and mites, built upon the collective knowledge of more than 80 taxonomic experts, we feel the data in Table 4 offer a unique look at the taxonomic distribution of ash-specialist herbivores from the estimated 70,000 species of North American insects (Arnett, 2000) and Acari (mites). Specialist herbivores that would be imperiled or extirpated in the United States and Canada by the loss of *Fraxinus* include mites (n = 6) and members of five insect orders: Lepidoptera (n = 32), Hemiptera (n = 25), Coleoptera (n = 24), Diptera (n = 9), and Hymenoptera (n = 3) (Fig. 21). The most speciose lineage of metazoans on the planet, beetles, had fewer specialists than Lepidoptera and essentially equivalent richness to that of Hemiptera. While the focal taxon of our study was *Fraxinus* and related Oleaceae, we suspect that the proportions represented here are likely to apply across most temperate woody, broadleaf plant taxa, and may well apply to other continental biogeographic provinces as well.

Four genera contain six or more species that will be threatened by the spread of EAB; in decreasing diversity these include *Tropidosteptes* plant bugs (Miridae) (n = 14) (Fig. 13), *Hylesinus* bark beetles (Curculionidae) (n = 7), *Lignyodes* seed weevils (Curculionidae) (n = 7) (Fig. 19), and *Sphinx* hawkmoths (n = 6) (Sphingidae) (Figs. 16, 17). Four other genera contain noteworthy radiations on *Fraxinus*: *Dasineura* gall midges (Cecidomyiidae), *Prociphilus* aphids (Aphididae), *Philtraea* inchworms (Geometridae) (Fig. 15), and *Sympistis* sallows (Noctuidae).
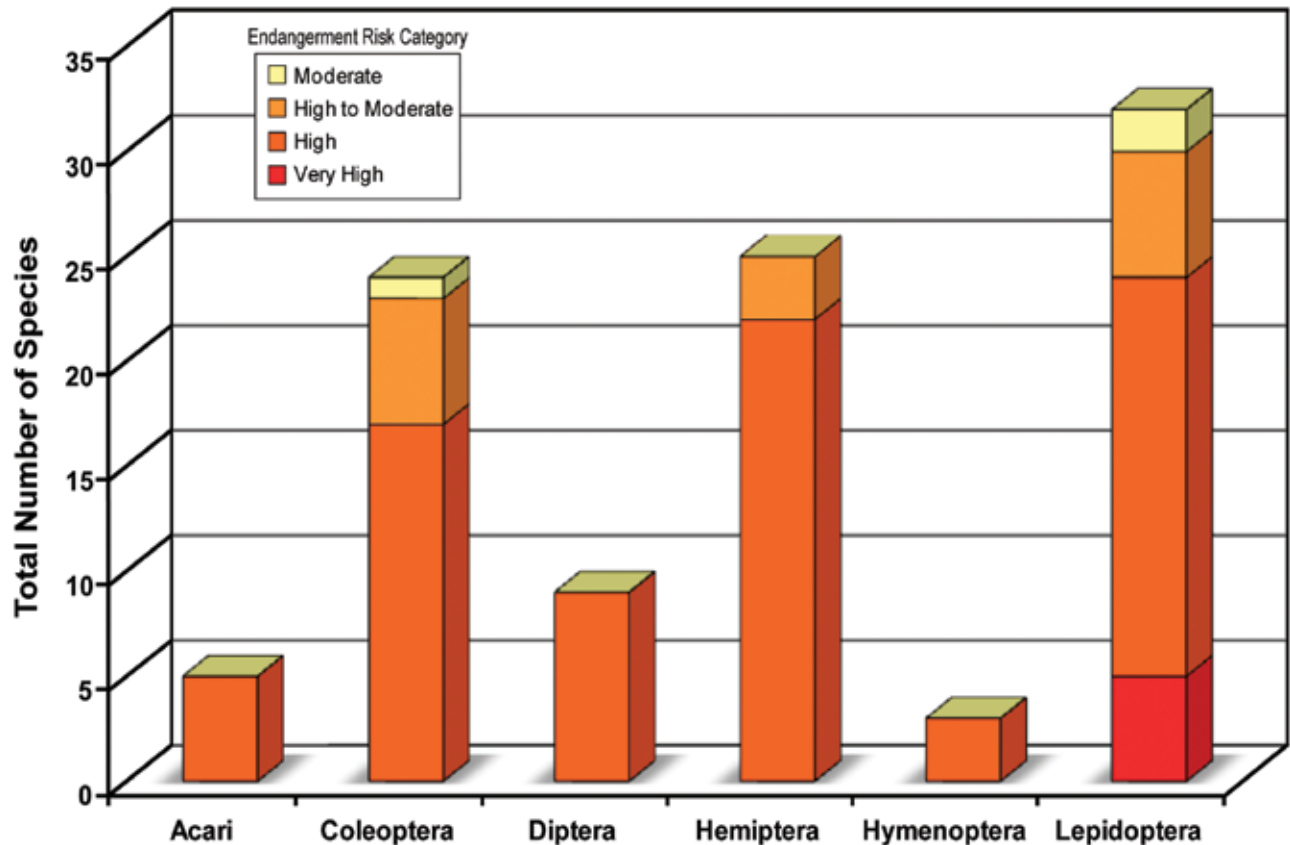
Figure 21. At risk arthropod herbivores by taxon and imperilment score.

Charismatic Arthropod Herbivores

The sphingids or hawkmoths deserve special mention because of their beauty, size, and popularity (with moth watchers, photographers, and collectors), as well as their vulnerability and importance in vertebrate diets. Furthermore, the degree of sphingid imperilment has heretofore been underestimated. Gandhi and Herms (2010) listed eight native and one exotic species of sphingids as potentially affected by EAB invasion; the only sphingid that they ranked as highly to moderately imperiled by EAB was *Manduca brontes*, a Caribbean and South Florida species that feeds on *Tecoma stans* (L.) Juss. ex Kunth in the Bignoniaceae. *Fraxinus* host records for the moth were determined to be erroneous by Robinson et al. (2002); we exclude this species from our assessment. Six sphingids that were regarded as generalists[3] of

low or moderate endangerment risk in their compilation are treated here as imperiled ash specialists: *Ceratomia undulosa*, *Manduca jasminearum*, *Sphinx canadensis*, *S. chersis*, *S. franckii* (Fig. 16), and *S. kalmiae* (Fig. 17). We add three resident hawkmoths not mentioned in previous ecological assessments of the North American sphingid fauna: *Ceratomia sonorensis*, *Sphinx libocedrus*, and *S.* near *chersis*. We assign endangerment risks to these nine ash-dependent

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[3] *Sphinx canadensis*, *S. chersis*, *S. kalmiae*, and *S. franckii*, *Manduca jasminearum*, and *Ceratomia undulosa* could be classified

as polyphagous taxa if one consults treatments such as Covell (2005), Wagner (2005), Tuttle (2007), Robinson et al. (2014), etc. But if records >50-years old or exceptional reports are excluded, all are better regarded as imperiled *Fraxinus* or Oleaceae specialists. Older literature is fraught with misidentifications and erroneous host records: For example, the host records of *Ulmus* for S. *franckii* and *M. jasminearum* are almost certainly based on misidentifications of larval *Ceratomia amyntor* (Geyer, 1835), an elm feeder. To the best of our knowledge (and that of Jim Tuttle in litt.) none of these six species has been collected on host plants outside of the Oleaceae in our lifetimes, and all but *S. kalmiae* (which can be found on other Oleaceae) are best considered *Fraxinus* specialists.

Table 4. Specialist herbivores on *Fraxinus* and related Oleaceae.

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Acari	Eriophyidae	*Aceria fraxiniflora* (Felt)	*Fraxinus americana, F. latifolia*	High	flower gall	Felt, 1918; Baker et al., 1996; Ron Ochua pers. comm.	
Acari	Eriophyidae	*Aceria fraxini* (Garman)	*Fraxinus americana, F. latifolia, F. pennsylvanica*	High	leaf gall	Felt, 1918; Baker et al., 1996	
Acari	Eriophyidae	*Tegolophus califraxini* (Keifer)	*Fraxinus dipetala, F. velutina*	High	leaves	Jeppson et al., 1975; Baker et al., 1996	
Acari	Tenuipalpidae	*Brevipalpus fraxini* DeLeon	*Fraxinus, F. profunda*	High	leaves	DeLeon, 1961; Baker and Suigong, 1988; Ron Ochua pers. comm.	
Acari	Tenuipalpidae	*Brevipalpus cardinalis* (Banks)	*Fraxinus velutina, F. albicans*	High	unknown	Banks, 1915; Baker and Suigong, 1988; Johnson, 2007	
Coleoptera	Buprestidae	*Agrilus subcinctus* Gory	*Fraxinus pennsylvanica, Ligustrum*	High to Moderate	cambium	Bright, 1987; Ted MacRae unpubl. data; Richard Westcott in litt.	
Coleoptera	Buprestidae	*Trigonogya reticulaticollis* (Schaeffer)	*Fraxinus berlandieriana*	High?	recently dead wood	MacRae, 2006; Ted MacRae unpubl. data; Richard Westcott in litt.	Confirmed larval host; Knull (1937) recorded adults collected on *F. caroliniana*, but that is a misidentification of *F. berlandieriana* (Richard Westcott in litt.). Host range needs more study.
Coleoptera	Chrysomelidae	*Capraita sexmaculata* (Illiger)	*Chionanthus virginicus, Fraxinus americana, F. berlandieriana, Forestiera ligustrina*	High to Moderate	leaves	Clark et al., 2004; Shawn Clark in litt.; Ed Riley in litt.	Probably obligatory on ash; adult diet includes other Oleaceae hosts.
Coleoptera	Chrysomelidae	*Trichaltica tibialis* (Jacoby)	*Fraxinus*	High	root	Clark et al., 2004; Shawn Clark in litt.; Ed Riley in litt.	Larvae root feeders; almost certainly specialists.
Coleoptera	Chrysomelidae	*Trichaltica scabricula* (Crotch)	*Fraxinus*	High	root	Clark et al., 2004; Shawn Clark in litt.	Larvae root feeders; almost certainly specialists.
Coleoptera	Curculionidae	*Hylesinus aculeatus* (Say)	*Fraxinus americana, F. pennsylvanica*	High	cambium	Blatchley and Leng, 1916; Wood, 1982; Atkinson, 2014, Anthony Cognato in litt.	

Table 4. (continued)

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Coleoptera	Curculionidae	*Hylesinus californicus* (Swaine)	*Fraxinus americana, F. latifolia, F. pennsylvanica, F. velutina, Olea europaea*	High to Moderate	cambium	Wood, 1982; Langor and Hergert, 1993; Atkinson, 2014; Anthony Cognato in litt.	
Coleoptera	Curculionidae	*Hylesinus criddlei* (Swaine)	*Fraxinus americana, F. pennsylvanica*	High	cambium	Atkinson, 2014; Anthony Cognato in litt.	
Coleoptera	Curculionidae	*Hylesinus fasciatus* LeConte	*Fraxinus americana*	High	cambium	Blatchley and Leng, 1916; Wood, 1982; Atkinson, 2014; Anthony Cognato in litt.	
Coleoptera	Curculionidae	*Hylesinus mexicanus* (Wood)	*Fraxinus albicans, Forestiera, Ligustrum, Olea*	Moderate	cambium	Atkinson, 2014; Anthony Cognato in litt.	
Coleoptera	Curculionidae	*Hylesinus oregonus* (Blackman)	*Fraxinus latifolia*	High	cambium	Wood, 1982; Burns and Honkala, 1990; Atkinson, 2014; Anthony Cognato in litt.	
Coleoptera	Curculionidae	*Hylesinus pruinosus* Eichhoff	*Fraxinus americana*	High	cambium	Wood, 1982; Atkinson, 2014; Anthony Cognato in litt.	
Coleoptera	Curculionidae	*Lignyodes arizonicus* (Sleeper)	*Fraxinus velutina*	High	seed	Clark, 1980; Robert Anderson pers. comm.	
Coleoptera	Curculionidae	*Lignyodes auratus* Clark	*Fraxinus latifolia*	High	seed	Clark, 1980; Robert Anderson pers. comm.	
Coleoptera	Curculionidae	*Lignyodes bischoffi* (Blatchley)	*Fraxinus americana, F. nigra, F. pennsylvanica, Syringa*	High to Moderate	seed	Knull, 1932; Clark, 1980; Robert Anderson pers. comm.	
Coleoptera	Curculionidae	*Lignyodes fraxini* (LeConte)	*Fraxinus americana*	High	seed	Clark, 1980; Robert Anderson pers. comm.	
Coleoptera	Curculionidae	*Lignyodes helvolus* (LeConte)	*Fraxinus americana, F. berlandieriana, F. nigra, Syringa*	High to Moderate	seed	Clark, 1980; Robert Anderson pers. comm.	

Table 4. (continued)

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Coleoptera	Curculionidae	*Lignyodes helvolus* (Le-Conte)	*Fraxinus americana, F. berlandieriana, F. nigra, Syringa*	High to Moderate	seed	Clark, 1980; Robert Anderson pers. comm.	
Coleoptera	Curculionidae	*Lignyodes horridulus* (Casey)	*Fraxinus latifolia, F. pennsylvanica, F. velutina, Syringa*	High to Moderate	seed	Clark, 1980; Robert Anderson pers. comm.	
Coleoptera	Curculionidae	*Lignyodes ocularis* (Casey)	*Fraxinus*	High	seed	Clark, 1980; Robert Anderson pers. comm.	
Coleoptera	Curculionidae	*Psomus armatus* (Dietz) (including *politus*)	*Fraxinus americana*	High	seed	Blatchley and Leng, 1916; Arnett et al., 2002; Charles O'Brien in litt.; Richard Westcott in litt.	
Coleoptera	Scarabaeidae	*Dynastes granti* Horn	*Fraxinus velutina*	High–see comments	new shoot feeder	Bill Warner in litt.; Pat Sullivan pers. comm.; Margarethe Brummerman pers. comm.	Larvae may be generalists but adults feed on ash; beetle rare or absent away from *Fraxinus* (Bill Warner in litt.).
Coleoptera	Scarabaeidae	*Dynastes tityus* L.	*Fraxinus*	High–see comments	new shoot feeder	Glaser, 1976	Adults feed (and mate?) at wounds gnawed into ash shoots.
Coleoptera	Scarabaeidae	*Xyloryctes jamaicensis* (Drury)	*Fraxinus americana*	High	root	Ratcliffe, 2009	See discussion in Ratcliffe 2009.
Coleoptera	Scarabaeidae	*Xyloryctes thestalus* Bates	*Fraxinus velutina* and likely other *Fraxinus* species, esp. in Mexico	High	root	Ratcliffe, 2009	See discussion in Ratcliffe 2009.
Diptera	Agromyzidae	*Phytobia* sp.	*Fraxinus americana, F. pennsylvanica*	High	cambium	Solomon, 1995; Sonja Sheffer in litt.	
Diptera	Cecidomyiidae	*Contarinia thalactri* (Felt) (including *fraxini*)	*Fraxinus, F. americana*	High	leaf gall (inquiline)	Gagné, 1989	Phytophagous inquiline in galls of *Dasineura tumidosae*.
Diptera	Cecidomyiidae	*Contarinia* n. sp.	*Fraxinus pennsylvanica*	High	samara gall	Gagné, 1989	Samara exocarp gall; gregarious; undescribed evidently; near European species *C. marchali.*

Table 4. (continued)

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Diptera	Cecidomyiidae	*Contarinia* n. sp.	*Fraxinus pennsylvanica*	High	seed or samara gall	Raymond Gagné in prep.	Seed or samara gall; undescribed; not near European species *C. marchali.*
Diptera	Cecidomyiidae	*Dasineura apicata* (Felt)	*Fraxinus americana*	High	leaflet gall	Felt, 1918; Gagné, 1989	
Diptera	Cecidomyiidae	*Dasineura fraxinifolii* (Felt)	*Fraxinus americana*	High	leaflet gall	Felt, 1918; Gagné, 1989	
Diptera	Cecidomyiidae	*Dasineura pellex* (Osten Sacken)	*Fraxinus americana*	High	leaflet gall	Felt, 1918; Gagné, 1989; Raymond Gagné in litt.	
Diptera	Cecidomyiidae	*Dasineura tumidosae* (Felt)	*Fraxinus americana*	High	leaflet gall	Gagné, 1989	
Diptera	Cecidomyiidae	*Dasineura* n. sp.	*Fraxinus pennsylvanica*	High	seed gall	Raymond Gagné in prep.	Reared from seed collection from Ontario (Raymond Gagné in prep.).
Hemiptera	Aphididae	*Prociphilus americanus* (Walker)	*Abies, Fraxinus dipetala, F. latifolia, F. velutina*	High	phloem	DeAngelis, 1998; TTD, 2014; Carol von Dohlen in litt.	*Abies* is alternate host.
Hemiptera	Aphididae	*Prociphilus fraxinifolii* (Riley)	*Fraxinus americana, F. nigra, F. velutina*	High	phloem	TTD, 2014; Carol von Dohlen in litt.	
Hemiptera	Aphididae	*Prociphilus pergandei* Smith	*Fraxinus americana*	High	phloem	Blackman and Eastop, 1994; Carol von Dohlen in litt.	
Hemiptera	Aphididae	*Prociphilus probosceus* (Sanborn)	*Fraxinus*	High	phloem	Blackman and Eastop, 1994; Carol von Dohlen in litt.	
Hemiptera	Diaspididae	*Diaspidiotus fraxini* (McKenzie)	*Fraxinus velutina*	High	phloem	Miller et al., 2014a; Douglass Miller in litt.	Few Diaspididae are specialists; confirmation of monophagy is needed.
Hemiptera	Diaspididae	*Diaspis fraxini* Ferris	*Fraxinus velutina* var. *tourneyi*	High	phloem	Miller et al., 2014b; Douglass Miller in litt.	Type locality about 42 miles from Arizona border. Uncertain if species has been reported from US; few Diaspididae are specialists (Ben Normark pers. comm.); confirmation of monophagy is needed.

Table 4. (continued)

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Hemiptera	Miridae	*Bisulcopsallus texanus* (Knight)	*Fraxinus*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes adustus* (Knight)	*Fraxinus americana*	High	phloem	Henry, 1980; TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes amoenus* (Reuter)	*Fraxinus americana, F. latifolia, F. pennsylvanica*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes brooksi* Kelton	*Fraxinus*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes canadensis* Van Duzee	*Fraxinus, F. pennsylvanica*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes cardinalis* Uhler	*Fraxinus americana*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes glaber* (Knight)	*Fraxinus americana*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes illitus* (Van Duzee)	*Fraxinus velutina*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes neglectus* (Knight)	*Fraxinus pennsylvanica*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes pacificus* (Van Duzee)	*Fraxinus latifolia, F. velutina*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes pettiti* Reuter	*Fraxinus*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes populi* (Knight)	*Fraxinus americana, F. pennsylvanica*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes rufvenosus* (Knight)	*Fraxinus*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes tricolor* Van Duzee	*Fraxinus pennsylvanica*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Miridae	*Tropidosteptes vittiscutis* (Knight)	*Fraxinus americana*	High	phloem	TTD, 2014; Michael Schwartz in litt.	
Hemiptera	Pentatomidae	*Banasa rolstoni* Thomas & Yonke	*Fraxinus velutina*	High	phloem	Donald Thomas pers. comm.	More study needed; other *Banasa sp.* are known to be generalists.

Table 4. (continued)

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Hemiptera	Tingidae	*Leptoypha costata* Parshley	*Chionanthus, Fraxinus*	High to Moderate	phloem	Usinger, 1945; Drake and Ruhoff, 1965; Tom Henry in litt; *Chionanthus* record from Laura Miller in litt.	*Carya ovata, Corylus,* and *Hamamelis* records for this species likely in error (Tom Henry in litt.).
Hemiptera	Tingidae	*Leptoypha minor* McAtee (including *nubilis*)	*Fraxinus latifolia, F. velutina,* and other Oleaceae	High to Moderate	phloem	Usinger, 1945; Drake and Ruhoff, 1965; Tom Henry in litt.	*Ceanothus, Populus* and other non-Oleaceae hosts in error (Tom Henry in litt.).
Hemiptera	Tingidae	*Leptoypha mutica* (Say)	*Chionanthus, Fraxinus americana*	High to Moderate	phloem	Froeschner, 1944; Usinger, 1945; Drake and Ruhoff, 1965; Tom Henry in litt; Laura Miller in litt	*Forestiera* records likely refer to another species (Tom Henry in litt.).
Hymenoptera	Tenthredinidae	*Eupareophora parca* (Cresson)	*Fraxinus americana, F. nigra, F. latifolia, F. pennsylvanica*	High	new leaves	Williams, 2007; David Smith in litt.	Pecan record is doubtful (David Smith in litt.).
Hymenoptera	Tenthredinidae	*Tethida barda* (Say)	*Fraxinus americana*	High	new leaves	David Smith in litt.	
Hymenoptera	Tenthredinidae	*Tomostethus multicinctus* (Rohwer)	*Fraxinus americana, F. latifolia*	High	new leaves	Langford and McConnell, 1935; David Smith in litt.	
Lepidoptera	Apatelodidae	*Olceclostera angelica* (Grote)	*Fraxinus, Syringa*	High to Moderate	mature leaves	Wagner, 2005, 2007; Robinson et al., 2014	
Lepidoptera	Geometridae	*Hydrelia* near *inornata*	*Fraxinus americana*	High	mature leaves	Wagner et al., 2002	Validity of this taxon in need of (molecular) verification.
Lepidoptera	Geometridae	*Philtraea elegantaria* (Hy. Edwards)	*Fraxinus gooddingii*	High	leaves	DLW unpubl. data	
Lepidoptera	Geometridae	*Philtraea latifoliae* Buckett	*Fraxinus latifolia*	Very High	leaves	Buckett, 1970; Wagner, 2007	Only known from a few counties in Central California and evidently declining.

Table 4. (continued)

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Lepidoptera	Geometridae	Philtraea paucimacula Barnes and McDunnough	Fraxinus greggii and Lycium	Moderate but see comments	leaves	Noel McFarland pers. comm.; Wagner unpubl. data	F. greggii record from Laredo, TX (Noel McFarland pers. comm.) and Big Bend by DLW; Lycium in Big Bend NP from Wagner unpubl. data but possibly two taxa involved in which case the ash feeder is imperiled.
Lepidoptera	Geometridae	Philtraea surcaliforniae Buckett	Fraxinus dipetala	High	leaves	Buckett, 1970; Wagner, 2007	
Lepidoptera	Geometridae	Plagodis kuetzingi (Grote)	Fraxinus americana, F. quadrangulata	High	leaves	Wagner, 2007	
Lepidoptera	Gracillariidae	Caloptilia fraxinella (Ely)	Fraxinus americana, F. nigra, F. pennsylvanica	High	new leaves	Johnson and Lyon, 1988; Pohl et al. 2004; Wagner, 2007	Ligustrum as a host is listed in error in previous tabulations; that record traces to C. cuculipennella (Charley Eiseman in litt.).
Lepidoptera	Gracillariidae	Caloptilia n. sp.	Fraxinus latifolia	High	new leaves	Wagner, 2007, unpubl. data	Found along creeks in central and northern California.
Lepidoptera	Gracillariidae	Marmara basidendroca Fitzgerald	Fraxinus pennsylvanica	Very High	bark miner that tunnels into root crown	Fitzgerald, 1973; Wagner, 2007	Currently known only from Upstate New York. Feeds on single cell layer in cork cambium.
Lepidoptera	Gracillariidae	Marmara corticola Fitzgerald	Fraxinus pennsylvanica	Very High	green bark miners	Fitzgerald, 1973; Wagner, 2007	Currently known only from Upstate New York. Feeds on single cell layer in cork cambium initially and then tunnels into deeper cork layers.
Lepidoptera	Gracillariidae	Marmara fraxinicola Braun	Fraxinus americana, F. pennsylvanica	High	green bark miners	Fitzgerald and Simeone, 1971; Fitzgerald, 1973; Wagner, 2007	Feeds on single cell layer in cork cambium.
Lepidoptera	Noctuidae	Chloronycta tybo (Barnes)	Fraxinus velutina	High	mature leaves	Schmidt et al., 2014	

Table 4. (continued)

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Lepidoptera	Noctuidae	*Copivaleria grotei* Morrison	*Fraxinus americana*	High	new leaves	Wagner, 2007; Robinson et al., 2014	
Lepidoptera	Noctuidae	*Papaipema furcata* (J. B. Smith)	*Fraxinus americana, F. nigra, F. pennsylvanica*	High	new shoot feeder (borer)	Wagner, 2007; Robinson et al., 2014	Non-*Fraxinus* hosts are insignificant, incidental, or erroneous (Eric Quinter in litt.). Larvae feed only in vigorous growing shoots usually of saplings.
Lepidoptera	Noctuidae	*Sympistis chionanthi* (J. E. Smith)	*Chionanthus virginicus, Fraxinus americana, F. nigra, F. pennsylvanica*	High to Moderate	new leaves	Wagner, 2007; Wagner et al., 2011; Robinson et al., 2014	*Triosteum* records likely in error and refer to *S. forbesi* (Zacharczenko et al. 2014).
Lepidoptera	Noctuidae	*Sympistis fortis* (Grote)	*Fraxinus, F. latifolia*	High	new leaves	Robinson et al., 2014; CDFA card file pest database	CDFA record for *Keckiella antirrhinoides* as a host likely derives from a misidentification of *S. picina*
Lepidoptera	Noctuidae	*Sympistis punctilinea* (Hampson)	*Fraxinus, F. velutina*	High	new leaves	Crumb, 1956; CDFA card file pest database	
Lepidoptera	Noctuidae	*Sympistis heterogena* (A. Blanchard)	*Fraxinus cuspidata*	High	new leaves	DLW unpubl. data	New leaves of *F. greggii* growing in Chisos Basin, Big Bend NP. Spring active.
Lepidoptera	Pyralidae	*Palpita magniferalis* (Walker)	*Fraxinus americana, F. nigra, F. pennsylvanica*	High	leaves	Robinson et al., 2014	
Lepidoptera	Sesiidae	*Podosesia aureocincta* Purrington & Nielson	*Fraxinus, F. americana, F. pennsylvanica, Olea*	High to Moderate	xylem	Eichlin and Duckworth, 1988; Wagner, 2007; Robinson et al., 2014	Sister to *syringae*: slight flight period and genitalic differences; COI barcode shared with *syringae*; reports from *Olea* should be verified.
Lepidoptera	Sphingidae	*Ceratomia sonorensis* Hodges	*Fraxinus velutina*	High	mature leaves	Tuttle, 2007; Wagner, 2005, 2007	
Lepidoptera	Sphingidae	*Ceratomia undulosa* (Walker)	*Chionanthus virginicus, Fraxinus american, F. caroliniana, F. nigra, F. pennsylvanica, Ligustrum, and Syringa*	High to Moderate	mature leaves	Tuttle, 2007; Wagner, 2005, 2007	DLW discounts all records other than those on Oleaceae: *Fraxinus* preferred host.

Table 4. (continued)

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Lepidoptera	Sphingidae	*Manduca jasminearum* (Guérin)	*Fraxinus, Syringa*	High	mature leaves	Tuttle, 2007; Wagner, 2005, 2007	DLW discounts all records other than those on Oleaceae.
Lepidoptera	Sphingidae	*Sphinx canadensis* Boisduval	*Fraxinus americana* (origin of this host requires verification) *F. nigra* (preferred host)	Very High	mature leaves	Tuttle, 2007; Wagner, 2005, 2007	DLW discounts all records other than those on Oleaceae. another *Fraxinus* species likely to be principal host species south of Ohio River.
Lepidoptera	Sphingidae	*Sphinx chersis* (Hübner)	*Fraxinus americana, F. pennsylvanica, F. velutina, Ligustrum, Syringa,* other Oleaceae	High to Moderate	mature leaves	Tuttle, 2007; Wagner, 2005, 2007	DLW discounts all records other than those on Oleaceae.
Lepidoptera	Sphingidae	*Sphinx near chersis*	*Fraxinus velutina*	High	mature leaves	Chris Schmidt in litt.	DNA barcodes suggestive that Arizona populations of *S. chersis* represent n. sp. sister to *S. mexicana* (Chris Schmidt in litt.)
Lepidoptera	Sphingidae	*Sphinx franckii* Neumoegen	*Fraxinus americana, Ligustrum*	Very High	mature leaves	Tuttle, 2007; Wagner, 2005, 2007	DLW discounts all records other than those on Oleaceae; already uncommon and declining; *Fraxinus* preferred host.
Lepidoptera	Sphingidae	*Sphinx kalmiae* J. E.	*Chionanthus virginicus, Fraxinus ameicana, F. nigra, F. pennsylvanica, Ligustrum, Syringa*	High to Moderate	mature leaves	Tuttle, 2007; Wagner, 2005, 2007	DLW discounts all records other than those on Oleaceae.
Lepidoptera	Sphingidae	*Sphinx libocedrus*	*Forestiera* spp., *Fraxinus gooddingii, F. greggii*	Moderate	mature leaves	Tuttle, 2007	*Forestiera* likely the principal host.
Lepidoptera	Yponomeutidae	*Zelleria near hepariella*	*Fraxinus latifolia*	High	leaves	Wagner, 2007	Report by Wagner (2007) as *Z. hepariella* in error; the use of *hepariella* traces to preliminary determination by Tom Eichlen (in litt.); taxonomy still under study.

Table 4. (continued)

Taxon	Family	Scientific Name	Confirmed Hosts	Risk Rating	Guild	Reference(s)	Comments
Lepidoptera	Yponomeutidae	*Zelleria near semitincta*	*Fraxinus dipetala*	High?	leaves	John DeBenedictis unpubl. data	A similar appearing moth occurs in southern California on *Forestiera*.

Figure 22. Mating pair of North America's largest beetle, *Dynastes granti* (Photo courtesy Margarethe Brummerman). So far as known, actively growing ash shoots are the principal feeding site for this behemoth, which sometimes exceeds 80 mm in length. Males chew a lesion into new growth and females are paired as they arrive at the wound. Curiously, its more widespread cousin, the Eastern Hercules beetle; *Dynastes tityus,* is virtually unstudied as an adult. The only adult feeding record of which we are aware, again is for a *Fraxinus* (Glaser 1976). Grant's rhinoceros beetle is quite sought-after by photographers and collectors; persons (even families) with interests in Coleoptera, sometimes travel from as far away as Japan and Korea to see the beetle. See also caption for Figure 23.

species as Very High (n = 2), High (n = 3), High to Moderate (n = 3), and Moderate (n = 1). Three of the nine have restricted global distributions and are ranked as G4 species in NatureServe Explorer (2014): *Manduca jasminearum*, *Sphinx canadensis*, and *S. franckii* (Fig. 16). The first of these is already in decline along the northern portion of its range (NatureServe Explorer, 2014), and sphingids (and especially members of the nominate genus *Sphinx*) have been identified as a group in decline across much of the northeastern United States, perhaps due to the exotic tachinid *Compsilura concinnata* (Meigen) (Wagner, 2012) or an as yet unidentified egg parasitoid (Sam Jaffe, unpublished data).

One of the most surprising findings from our study is that one of North America's largest, and

attention-worthy insects may be threatened by EAB: the western rhinoceros beetle (*Dynastes granti*), whose adults occasionally exceed 80 mm in length (Figs. 18, 22). While this large scarab is a generalist as a larva, feeding in dead wood of various tree species, its adult may prove to be a specialist that feeds primarily on the new growth of velvet ash in Arizona. Courting males chew into the cambial layers of an ash tree and then await the arrival of females (which presumably feed and mate at the wound site). The best known and most reliable locations for the beetle occur in the vicinity of velvet ash, where the adults have access to vigorously growing green shoots (Margarethe Brummerman, Patrick Sullivan, and Bill Warner, all in litt.). Beetle collectors visit the southwestern United States with

the Grant's rhinoceros beetle as a common focus of their itineraries. Rhinoceros beetles are also popular pets in some Asian countries; we know of instances of Asian families traveling to Arizona primarily to acquire living *Dynastes* adults to keep as pets and captive breeding (Fig. 23). *Dynastes granti* is even occasionally sold in Japanese pet shops and has appeared in staged beetle fights.

Less is known about the habits and dietary proclivities of the eastern rhinoceros beetle (*Dynastes tityus*), but it too is known to chew into shoots of ash and feed at the wounds (Glaser, 1976); no other adult food plants are known to us. Ratcliffe (2009) called attention to the threat of EAB to two other rhinoceros beetles in the genus *Xyloryctes*, which reach lengths close to 30 mm (Fig. 20). Both North American species are specialists on ash roots as larvae. If even a few of the species mentioned above were to disappear, the losses to North American arthropod fauna would be great (in both senses of the word).

Figure 23. Entomophily. The insect zoo at the United States Museum of Natural History, ranks only behind dinosaurs as a public draw. Insects also enjoy considerable favorable attention in many Asian countries. This photo is from an insect exhibition held in the Tokyo Tower over a six-week span in 2013 (http://www.japantrends.com/tokyo-tower-insect-exhibition/) (Photo courtesy of Tokyo Tower, Nippon Television City Corporation). More than 16,000 insects were on display, many of which were alive in the featured insect jungle. The scene above shows a beetle enclosure with many horned scarabs, kindred to and perhaps even including the North American *Dynastes* and *Xyloryctes* that are listed in Table 4 and shown in Figures 18, 20, and 22. Asian families are also known to travel annually to the Southwest United States to look for live *D. granti* adults to be kept as family pets and bred.
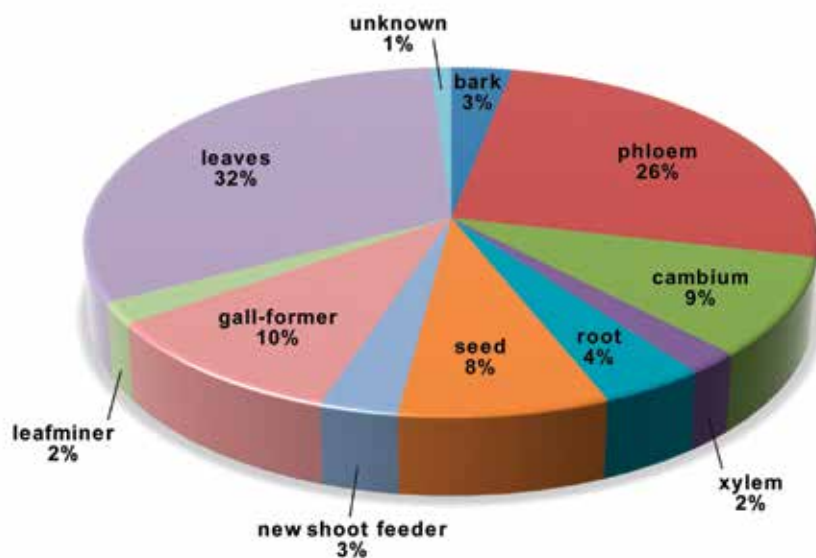
Figure 24. Feeding guilds of specialist herbivores on ash. Chart shows 98 species of ash specialists divided by feeding guild: bark (3), phloem (26), cambium (9), xylem (2), root (4), seed (8), new shoot feeder (3), gall-former (10), leaf miner (2), leaves (32), and unknown (1). Some species could be included into two feeding guilds, e.g., gall formers may form galls on seeds or leaves, etc. In these cases, we grouped individuals into the most applicable guild. See Table 4 for additional notes on feeding guilds.

Feeding Guilds and Specificity of Arthropod Herbivores

We group the 98 phytophagous arthropods identified in Table 4 into ten feeding guilds (Fig. 24). The degree of host specificity for the majority of the species listed in Table 1 is not known in detail. We are unaware of any taxonomically comprehensive study of ash herbivores where researchers thoroughly sampled across the spectrum of available *Fraxinus* (and other Oleaceae). It is our belief that most *Fraxinus* specialists (as defined in this work) consume a spectrum of available ash species growing in a given locale. Stated differently, we see little indication that the phylogenetic, physiological, and morphological differences across the genus *Fraxinus* are so great that ash-specialized herbivores are routinely restricted to a single species or even Section of the genus (contrary to what one might be led to believe from published host records). In the western United States many of the herbivores listed in Table 4 are associated with a single ash species simply because only one *Fraxinus* (or member of the Oleaceae) occurs at a given locale. In eastern North America, in cases where only *Fraxinus* (without a species indicated) is listed in Table 4, the host is likely assignable to *F. americana* and/or *F. pennsylvanica*. Based on our literature review, communications with >80 taxonomists, and DLW's 30-year rearing program of North American Lepidoptera (that has encompassed more than 2,000 species representing more than 50 families, including miners, gall-formers, wood feeders, and others), the following generalities emerged.

Wood feeders. As reflected in our results, wood feeders (restricted here to taxa consuming mostly subcambial tissues including dead and live wood feeders) are seldom specialized in diet, e.g., not one of North America's 1000 cerambycids is known to be a *Fraxinus* specialist as a larva. We list only two wood feeders: the buprestid, *Trigonogya reticulaticollis*, whose host range is not well investigated and the clearwing borer *Podosesia aureocincta,* which is likely oligophagous on Oleaceae.

Phloem feeders. We identify 25 *Fraxinus* specialist, phloem-feeding hemipterans. While several of these appear to be monophagous, we believe that this is a sampling artifact and that the species in Table

Figure 25. *Marmara fraxinella*, Family Gracillariidae (Photo courtesy Charley Eiseman). Leafminers of the genus *Marmara* are specialized herbivores. Three North American *Marmara* are known only to feed on *Fraxinus*, and two of these mine only in the bark of young, actively growing ash trees.

Figure 26. Four males of the two-tailed swallowtail (*Papilio multicaudata*), Arizona's state butterfly, puddling in Oak Creek canyon, outside of Sedona, Arizona. Larvae feed on members of three plant families, but only ashes are present in some of the western canyons where this large swallowtail butterfly occurs. (Photo courtesy Tyger Gilbert, www.TygerGilbert.com)

4 will be found to feed on multiple species of ash. The aphids (n = 3), diaspidid scales (n = 2), and mirids (n = 15) appear to be obligate *Fraxinus* feeders. Tom Henry (in litt.) believes that the three tingids listed in Table 4 are oligophagous on Oleaceae and that contrary host records are unreliable. The diet breadth of the pentatomid *B. rolstoni* requires further study as very few stink bugs are known to be host plant specialists.

Cambium feeders. We record seven bark beetles and a cambium-mining agromyzid (*Phytobia* sp.) as ash specialists. The only buprestid identified as an ash specialist – from a family with more than 760 North American species – was *Agrilus subcinctus*, a cambium miner (of course, we exclude *A. planipennis*).

As a subcategory to cambium feeders, we include the three gracillariids that mine in green bark. *Marmara basidendroca* tunnels into cambial layers at least for part of its life cycle (Fitzgerald, 1973). This gracillariid and two of its congeners, *M. corticola* and *M. fraxinicola* are all highly specialized insects regarded to be at high risk (Fig. 25).

New shoot feeders. We include the borer *Papaipema furcata* (Noctuidae), which so far as is known, tunnels only in new growth of Melioides *Fraxinus*. Both western and eastern rhinoceros beetles (*D. granti* and *D. tityus*) can be provisionally placed here because adults are only known to feed at vigorously growing shoots of ash.

Root borers. We report four coleopterans whose larvae are ash root specialists – two leaf beetles: *Trichaltica tibialis* and *T. scabricula* and two scarab beetles: *Xyloryctes jamaicensis* and *X. thestalus* (Fig. 20). The extent to which the larvae of these beetles accept different species of ash has not been studied, although their ranges are broad enough to suggest that none is monophagous.

Seed feeders. Members of this guild, or at least the weevils of the genus *Lignyodes* (Fig. 19), appear to be oligophagous. *Psomus armatus,* so far known only from white ash, is expected to feed on other ashes based on what is known of the diet breadth of related weevils.

Gall formers. Gall insects are widely recognized to be among the most specialized insect herbivores (Felt, 1918, Gagné, 1989). The two gall-forming eriophyid mites that we list have been reported from more than one species of Section Melioides ashes. Gagné (in litt.) believes that all the cecidomyiid gall formers in Table 1 will be found to occur on more than one species within a *Fraxinus* Section, but that significant differences are likely to exist among Sections.

Leafminers. There are only two leafminers in our study, both members of the genus *Caloptilia* (Gracillariidae). One leafminer, *Caloptilia* n. sp., is an ecological specialist on *F. latifolia*; its eastern sister taxon, *C. fraxinella*, feeds on at least three species from two *Fraxinus* sections.

External (chewing) leaf feeders. Only one externally feeding lepidopteran is recorded as monophagous: *Philtraea latifoliae* (Geometridae), and

its dependency on Oregon ash is likely a geographical artifact because its host is the only member of the Oleaceae that grows where the moth occurs. One surprising outcome of our compilation is how proportionately few specialist external leaf feeders were detected (<32% of the 98 *Fraxinus*-dependent herbivores), though this guild comprises most of the insect biomass using ash and the most conspicuous herbivores on *Fraxinus*. Clearly, the insects that live and feed inside seeds, stems, or leaves dominate our list of species threatened by EAB.

Oligophages, Polyphages, and Other Trophic Levels

Our ecological assessment focused on specialists; it ignored oligophages and polyphages that might be affected by ash decline. However, over the course of preparing this manuscript, we discovered a few instances where an oligophage not treated in our list might be affected by EAB. For example, the two-tailed swallowtail (*Papilio multicaudatus*) (Fig. 26) uses *Fraxinus, Prunus,* and *Ptelea* as hosts in arid lands of western North America, but only ash species are available in some canyons where this butterfly lives. *Octotoma* (hispine chrysomelids), e.g., *O. plicatula* and *O. marginicollis*, were not included because their leafmining larval stages are specialists on non-ash species, even though the adult beetles are believed to do much of their adult feeding on *Fraxinus* species (Shawn Clark, in litt.).

We did not find mention of any specialist parasitoids, predators, or pathogens that we could confidently state were dependent on an ash herbivore. The recently described *Mymaromella pala* Huber & Gibson (Mymarommatidae) is known only from ash log collections, but its presumed host is a bark-residing psocid (Huber et al., 2008) likely to dwell on other trees as well. Gagné (1989) regards *Contarinia thalactri* to be a phytophagous inquiline in the galls of *Dasineura tumidosae*. However, beyond this inquiline record, we did not find mention of any other indirectly ash-specialized insects.

DISCUSSION

Imperilment Risk Ratings

It is our estimate that no less than 98 species of invertebrate herbivores would be appreciably affected by an elimination or massive reduction in abundance of North America's 16 native *Fraxinus*. For these herbivores we estimate endangerment risks as Very High (n = 5), High (n = 75), High to Moderate (n = 15), and Moderate (n = 3) (Fig. 21). Forty-five of the species-level taxa listed in Table 4 are identified as "at risk" for the first time. Eighteen (6%) of the taxa identified by Gandhi and Herms (2010) had their imperilment status upgraded; twenty six (35%) of the species listed in their treatment as being of high to moderate endangerment risk are dropped from consideration (Appendix 3). A key message deriving from this expert-based approach is that researchers should use caution when gleaning host records from dated literature, the internet, and especially secondary and tertiary resources. We found records (and especially older host records) to be rife with invalid taxa, erroneous identifications, instances where a presumed ash specialist was not phytophagous, cases where presumed ash specialists were generalists (type I errors), cases where presumed oligophagous or generalist herbivores were in fact specialists (type II errors), and cases where exotic or extralimital taxa were included.

As noted previously, we discounted the ecological importance of introduced (exotic) plants, e.g., lilac, privet, and olive, as alternative hosts that could support sufficiently large populations to ensure a taxon's long-term survival in North America. This seems to be a defensible position if one were to invoke the precautionary principle in assessing risk. But, we also recognize that it is possible that some invasive plants (such as *Ligustrum*) could play a role in the survival of some native Oleaceae specialists were EAB to eliminate much of the *Fraxinus* in a region.

The Very High risk rating – given to just five lepidopterans – was reserved for ash specialists believed to be at risk or in decline due to other causes. *Marmara basidendroca* and *M. corticola* (both Gracillariidae) are both specialized stem

miners known only from a restricted area in Upstate New York. *Philtraea latifoliae* (Geometridae) feeds exclusively on *Fraxinus latifolia* and is known only from a few counties in central California (Buckett, 1970). *Sphinx canadensis*' rating was raised to Very High because it is principally associated with *F. nigra*, an ash that is highly susceptible to EAB infestation (Leah Bauer pers. comm.) and which is predicted to be increasingly at risk due to climate change (Liang and Fei, 2014). *Sphinx franckii* is already uncommon and northern populations are in decline (Wagner, 2012).

For perspective on the importance of ash and the emerald ash borer, it is useful to compare ash decline with what is known about American chestnut (*Castanea dentata* [Marsh.] Borkh.) and its dependent herbivore fauna. Opler (1978) listed seven species of Lepidoptera that may have gone extinct as a result of losing this once ecologically dominant forest tree. One of these seven moths has since been rediscovered (*Synanthedon castaneae* [Busck]) (Anagnostakis et al., 1994), and another listed species, *Tischeria perplexa* Braun, may not be a valid species given that other Fagaceae-feeding *Tischeria* from the eastern United States are not chestnut specialists (Braun, 1972)[4]. Thus, five is a better estimate of the number of moth species that have been lost from the North American fauna[5] due to American chestnut decline. If one compares the number of extinct moths restricted to chestnut (n = 5) to the at risk ash-feeding Lepidoptera listed in Table 4 (n = 32), one is immediately struck by the magnitude of the threat posed by the introduction and spread of EAB. North America has not faced a threat of this magnitude to its native insect herbivore biodiversity from an exotic species over the course of the last two centurie. We believe that this is because

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[4] Three other *Tischeria* occur on *Castanea dentata* – one of which, *T. castaneaeella*, is believed to be quite closely related to *T. perplexa*, the purported *Castanea dentata* specialist. All three of the extant *Tischeria* that fed on chestnut are breeding on red oaks. Given the above, it is DLW's belief that *T. perplexa* will prove to be a taxonomic synonym.

[5] As a caveat, it is worth noting that no one has systematically sampled introduced *Castanea* or related native *Castanea*, and especially Alleghany chinquapin (*C. pumila*), which co-occurred with American chestnut; nor have workers surveyed nut-producing stands of *C. dentata* in Ohio, Maine, and elsewhere for the missing species.

Fraxinus represents a phylogenetically and or chemically isolated biological island that is too far removed from its allied genera (e.g., *Chionanthus*, *Forestiera*, exotic *Ligustrum*, and Oleaeceae) for these plants to serve as refuges for *Fraxinus* specialist herbivores over ecological and evolutionary time.

Data Gaps

Host ranges for virtually all of the taxa in this study remain incompletely known. Targeted herbivore surveys of the 16 North American *Fraxinus* would be valuable, especially for understudied ash species and those currently under threat from EAB or soon to be attacked as the beetle's range expands. In particular, southern ash are poorly studied as they tend to grow in swamps and all nine western ash species require further research; almost nothing is known of *F. albicans*, *F. berlandieriana*, *F. cuspidata*, *F. gooddinggii*, and *F. papillosa*. Likewise, the importance of other native Oleaceae, especially *Forestiera*, as hosts for ash-feeding herbivores is in need of study. Of critical importance to the evaluation of imperilment risk is the possibility that other native Oleaceae may be susceptible to the emerald ash borer. Only very recently did researchers learn that *Chionanthus* is also susceptible to attack (Entomology Today, 2014). Other Oleaceae species such as *Osmanthus* Lour., a genus of trees found in black water streams of the southern United States, remain virtually unstudied. While the susceptibility of other native Oleaceae to EAB is of no commercial relevance, it is a matter of considerable conservation significance.

We suspect that modern systematic analyses employing molecular markers will reveal additional cryptic species that are ash specialists (especially in the western United States where species-level taxonomic studies lag). One interesting example that surfaced over the course of our studies involves the sizable and charismatic great sphinx (*S. chersis*). COI barcodes suggest the hawkmoth is two species, with the populations from southeastern Arizona representing an as yet undescribed species that Chris Schmidt (in litt.) believes is closely related to *S. mexicana*. Conversely, some taxa in our treatment may have been over split taxonomically. The mirid genus with 14 *Tropidosteptes* species is in need of

revision, i.e., some names in Table 4 may prove to be synonyms (Michael Schwartz pers. comm.).

In summary, we recognized 98 species of herbivores as being threatened by the loss of *Fraxinus* in the United States and Canada. No doubt, additional ash specialists will be identified in the coming decades. Knowledge of herbivores feeding on ash is severely lacking in the southern and western United States; very little is known of the phytophagous insects feeding on six western *Fraxinus* species (*F. anomala*, *F. cuspidata*, *F. dipetala*, *F. gooddingii*, *F. greggii*, and *F. papillosa*). Gall midges (Cecidomyiidae), curculionid weevils, mites, thrips, and other taxonomically challenging arthropods can be expected to yield additional *Fraxinus* specialists. The latter two taxa seem especially likely to include ash specialists because their taxonomy is nascent and no systematic continent-wide surveys have been carried out. Across all taxa, molecular markers can be expected to reveal new cryptic specialist herbivores, especially in those taxa where the species-level taxonomy has proven difficult.

CONCLUSION

For the purposes of our ecological assessment we embraced the precautionary principle. The body of our assessment is, at its essence, a doomsday scenario: what might transpire if North America lost all of its *Fraxinus* or if ash numbers dwindled to a point where ash lost functional value in North American wildlands. Presently, EAB is spreading at a dramatic pace. We hope a growing number of parasitoids, pathogens, and predators will soon reduce the beetle's hyperabundance and rate of spread, and that some subset of ash species, genotypes, age classes, etc. will prove resistant to EAB. However, should the beetle cause catastrophic losses of ash, as many as 150 U.S. plant community types (16 of which are regarded as imperiled or critically imperiled) could be severely compromised. *Fraxinus* has a surprisingly rich, specialized, beautiful, and noteworthy invertebrate fauna, including hercules beetles, rhinoceros beetles, and hawkmoths – some of our continent's most magnificent invertebrates. By our assessment, the magnitude of North American invertebrate

biodiversity loss could greatly exceed that associated with American chestnut blight. The number of plant communities likely to be affected, the number of herbivores at risk (nearly 100 species), and the charismatic nature of the fauna in peril, argue for continued and increased efforts to bring the emerald ash borer under control.

ACKNOWLEDGMENTS

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APPENDIX 1. Contacted forest managers, plant ecologists, land managers, and wildlife biologists

State	Contact	Affiliation	Area of Expertise/Comments
Connecticut	May, Dale	CT-Department of Environmental Protection (DEEP)	retired wildlife biologist; specialist on game species
Delaware	Tallamy, Doug	University of Delaware	Professor of Entomology and Wildlife Ecology; author of "Bringing Nature Home"
Indiana	Dunbar, Richard	Indiana Department of Natural Resources (IND-NR)	Northeast Region Ecologist; Division of Nature Preserves
	Homoya, Mike	Indiana Department of Natural Resources (IND-NR)	Botanist; Division of Nature Preserves
	Shuey, John	The Nature Conservancy (TNC)	Director of Conservation Science
Michigan	Kashian, Dan	Wayne State University	Professor of Biological Sciences; forest ecologist
	McCullough, Deborah	Michigan State University	Professor of Entomology; forest entomologist
	Reznicek, Anton	University of Michigan	Assistant Director of University of Michigan Herbarium; plant systematist; plant ecologist
Ohio	Bissell, James	Cleveland Museum of Natural History	Curator of Botany; Coordinator of Natural Areas (50+ preserves)
	Boone, Daniel	Bartlett Tree Experts	Certified arborist and field botanist
	Hausman, Constance	Cleveland Metropolitan Park District	Plant and Restoration Ecologist
	Gardener, Richard	Ohio Department of Natural Resources (ODNR)	Chief Botanist (ODNR); ecologist
	McCormac, James	ODNR- Ohio Division of Wildlife	non-game wildlife biologist
	Reinier, John	Cleveland Metropolitan Park District	Wetland Ecologist
	Semroc, Judy	Cleveland Museum of Natural History	Conservation Specialist
Ontario	Catling, Paul	Agriculture and Agri-food Canada	Research Scientist; botanist
	Gill, Bruce	Canadian Food Inspection Agency	Research Scientist; entomologist; invasive species biologist
	Oldham, Michael	Ontario Natural Heritage Information Centre (NHIC)	Botanist; herpetologist
	Waldron, Gary	University of Guelph	School of Environmental Sciences; tree specialist
Wisconsin	Wilterding, John	Olivet College	Professor of Biology and Chair of the Department of Natural and Physical Sciences; entomologist

APPENDIX 2. Contacted taxonomic authorities and principal experts for arthropod records.

Taxon	Family	Taxonomic Authority	Affiliation	Position/Area of Expertise
Acari	Eriophyidae	Ochua, Ron	Systematic Entomology Lab, USDA Agriculture Research Service (ARS)	Research Entomologist
Acari	Tetranychidae	Ochua, Ron	Systematic Entomology Lab, USDA Agriculture Research Service (ARS)	Research Entomologist
Coleoptera	Brentidae	LaBonte, James	Insect Museum, Oregon Department of Agriculture	Museum Curator; Lead Taxonomic and Survey Entomologist
Coleoptera	Buprestidae	MacRae, Ted	Monsanto Company	Senior Research Entomologist & Project Leader
		Wescott, Rick	Insect Pest Prevention & Management Program; Oregon Department of Agriculture	Research Entomologist emeritus
Coleoptera	Cerambycidae	Alten, Ron	N/A	expert on western fauna
		Lingafelter, Steven	Systematic Entomology Lab, National Museum of Natural History, Smithsonian Institution	Research Entomologist
		MacRae, Ted	Monsanto Company	Senior Research Entomologist & Project Leader
Coleoptera	Chrysomelidae	Clark, Shawn	Monte L. Bean Life Science Museum, Brigham Young University	Entomology Collections Manager
		Riley, Ed	Texas A&M University Insect Collection	Associate Curator
		Staines, Charles	Department of Entomology, National Museum of Natural History, Smithsonian Institution	Research Entomologist
		Sullivan, Patrick	The Haden Collection, Sierra Vista, AZ	Executive Curator
Coleoptera	Curculionidae	Anderson, Robert	Canadian Museum of Nature	Research Scientist
		Atkinson, Thomas	Insect Collection, University of Texas, Austin	Collections Manager
		Burke, Horace R.	Department of Entomology, Texas A&M University	Professor emeritus
		Clark, Wayne	Department of Entomology and Plant Pathology, Auburn University	Professor emeritus
		Cognato, Anthony	A.J. Cook Arthropod Research Collection, Michigan State University	Associate Professor of Insect Systematics & Research Collection Director
		O'Brien, Charles W.	University of Arizona	Research Entomologist
Coleoptera	Hispinae	Staines, Charles	Department of Entomology, National Museum of Natural History, Smithsonian Institution	Research Entomologist

APPENDIX 2. (continued)

Taxon	Family	Taxonomic Authority	Affiliation	Position/Area of Expertise
Coleoptera	Scarabaeidae	Ratcliffe, Brett	Systematic Research Collections, University of Nebraska State Museum	Collection Curator, Professor of Entomology
		Sullivan, Patrick	The Haden Collection, Sierra Vista, AZ	Executive Curator
		Warner, Bill	California State Collection of Arthropods, California Department of Food and Agriculture (CDFA)	Research Associate
Coleoptera	various	Hespenheide, Henry	Department of Ecology and Evolutionary Biology, University of California Los Angeles (UCLA)	Professor Emeritus
		Quinn, Mike	affiliate of Texas A&M, University of Texas at Austin	expert on Texas fauna
		Schiff, Nathan	Center for Bottomland Hardwoods Research, USDA Forest Service	Research Entomologist
Diptera	Agromyzidae	Scheffer, Sonja Jean	Systematic Entomology Lab, USDA Agriculture Research Service (ARS)	Molecular Systematist
Diptera	Cecidomyiidae	Gagné, Raymond	Systematic Entomology Lab, National Museum of Natural History, Smithsonian Institution	retired Research Entomologist, current Collaborator
Hemiptera	Alyerodidae	Creel, Debra	USDA Agriculture Research Service (ARS)	Museum Specialist
Hemiptera	Aphididae	Blackman, Roger	Natural History Museum, London	Scientific Associate
		Miller, Douglass	Systematic Entomology Lab, USDA Agriculture Research Service (ARS)	retired Research Entomologist, current Collaborator
		von Dohlen, Carol	Utah State University	Professor of Biology
Hemiptera	Cicadellidae	McKamey, Stuart	Systematic Entomology Lab, National Museum of Natural History, Smithsonian Institution	Research Entomologist
Hemiptera	Coccoidea	Normark, Benjamin	University of Massachusetts, Amherst	Professor of Biology
		Watson, Gillian	California Department of Food and Agriculture (CDFA)	Senior Insect Biosystematist
Hemiptera	Derbidae	O'Brien, Lois	University of Arizona	systematist
		Wilson, Stephen	University of Central Missouri	Professor of Biology

APPENDIX 2. (continued)

Taxon	Family	Taxonomic Authority	Affiliation	Position/Area of Expertise
Hemiptera	Miridae	Schwartz, Michael	Eastern Cereal and Oilseed Research Centre, Agriculture and Agri-Food Canada	Research Entomologist
Hemiptera		Henry, Thomas	Systemic Entomology Lab, National Museum of Natural History, Smithsonian Institution	Research Entomologist
		Wheeler, Al	Clemson University	Adjunct Professor of Entomology
Hemiptera	Pentatomidae	Rider, Dave	North Dakota State University	Professor of Entomology
		Thomas, Donald	Subtropical Agriculture Research Center, USDA Agriculture Research Service (ARS)	Research Entomologist
Hemiptera	Psyllidae	Miller, Douglass	Systematic Entomology Lab, USDA Agriculture Research Service (ARS)	retired Research Entomologist, current Collaborator
Hemiptera	Tingidae	Henry, Thomas	Systemic Entomology Lab, National Museum of Natural History, Smithsonian Institution	Research Entomologist
		Miller, Laura	Pest Identification Laboratory, West Virginia Department of Agriculture	Taxonomic Entomologist
		Wheeler, Al	Clemson University	Adjunct Professor of Entomology
Hemiptera	various	Bartlett, Charles	University of Delaware	Associate Professor of Entomology and Wildlife Ecology
Hymenoptera	Tenthredinidae	Smith, David	Systemic Entomology Lab, National Museum of Natural History, Smithsonian Institution	retired Research Entomologist, current Collaborator
Hymenoptera	Eurytomidae	Gates, Michael	Systemic Entomology Lab, National Museum of Natural History, Smithsonian Institution	Research Entomologist
Lepidoptera	Geometridae	McFarland, Noel	N/A	authority on life histories of western moths
		Rand, Evan	N/A	Arizona moth expert
Lepidoptera	Gracillariidae	Davis, Don	National Museum of Natural History, Smithsonian Institution	Curator
Lepidoptera		Fitzgerald, Terry	State University of New York (SUNY) at Cortland	Distinguished Professor of Environmental Sciences and Forestry
Lepidoptera	Noctuidae	Quinter, Eric L.	Follett Institute of Natural Sciences; University of Connecticut affiliate	retired collection manager

APPENDIX 2. (continued)

Taxon	Family	Taxonomic Authority	Affiliation	Position/Area of Expertise
Lepidoptera	Pyralidae	Hayden, James	Florida State Collection of Arthropods, Florida Department of Agriculture	Taxonomic Entomologist, Lepidoptera Curator
Lepidoptera	Sesiidae	Hansen, Jason	Michigan State University	Research Scientist
Lepidoptera	Sphingidae	Schmidt, Chris	Canadian National Collection of Insects Canadian Food Inspection Agency, Ottawa	systematist with Canadian Food Inspection Agency
Lepidoptera		Tuttle, James P.	N/A	taxonomic expert
Lepidoptera	Yponomeutidae	DeBenedictis, John	Bohart Museum, University of California Davis	Research Fellow
Lepidoptera		Epstein, Marc	Plant Pest Diagnostics Center, California Department of Food and Agriculture	Senior Insect Biosystematist
		Landry, Jean-Francois	Canadian National Collection of Insects, Arachnids and Nematodes	Taxonomic Entomologist
		Powell, Jerry	Essig Museum of Biodiversity, University of California- Berkley	Director Emeritus
		Sohn, Jay	Department of Entomology, National Museum of Natural History, Smithsonian Institution	Post-Doctoral Fellow
Lepidoptera	various	Jump, Peter	N/A	taxonomic expert
Lepidoptera	various	Wagner, David	University of Connecticut	Professor of Ecology and Evolutionary Biology
Leafminers and Gall-Formers	various	Eiseman, Charley	N/A	taxonomic expert
Thysanoptera	various	Creel, Debra	USDA Agriculture Research Service (ARS)	Museum Specialist
Thysanoptera	various	Hoddle, Mark	Department of Entomology, University of California at Riverside	taxonomic expert
Thysanoptera	various	Mound, Laurence	Ecosystem Sciences, Commonwealth Scientific and Industrial Research Organisation (CSIRO)	Honorary Research Fellow

APPENDIX 3. Misattributions from Published Literature and Online Sources

Taxon	Species	Previous Ranking	Current Ranking	*Fraxinus* Specialist?	Taxonomically Valid?	Herbi-vore?	Native to U.S.?	Comment(s)
Acari	*Eriophyes fraxini* Garm.	High	High		Invalid Taxonomy			Valid name: *Aceria fraxini* (Baker et al., 1996)
Acari	*Tetranychus homorus* Pritchard & Baker	High to Moderate	Not Imperiled	Misattributed *Fraxinus* Specialist				Polyphagous; hosts include *Carya, Fraxinus, Gossypium*, and *Poaceae* (INRA, 2014)
Coleoptera	*Anthicus nitidulus* LeConte	Moderate	Not Imperiled	Misattributed *Fraxinus* Specialist	Invalid Taxonomy	Not an Herbivore		Valid name: *Ischyropalpus nitidulus*; mite predator (Landwer, 1977)
Coleoptera	*Apion porosicolle* Gemm.	High	Not Imperiled	Misattributed *Fraxinus* Specialist	Invalid Taxonomy			Valid name: *Apion cribricolle*; recorded on deer weed, deer vetch, wild buckwheat and *Olea* (Bright, 1993)
Coleoptera	*Lytta sphaericollis* Say	Moderate	Not Imperiled	Misattributed *Fraxinus* Specialist	Invalid Taxonomy			Valid name: *Linsleya sphaericollis*; larvae feed on grasshopper eggs, adults on *Fraxinus*, lilac and honey suckle (Church and Gerber, 1977; Pinto and Bologna, 1999)
Coleoptera	*Obrium rufulum* Gahan	Moderate	Not Imperiled	Misattributed *Fraxinus* Specialist				No North American cerambycids are known to be ash specialists (Ted McCrae in litt.)
Coleoptera	*Octotoma plicatula* (Fabricius)	High to Moderate	Further Research Required	Misattributed *Fraxinus* Specialist				Larvae feeds on *Campsis radicans* (Ed. Riley in litt.). Adults oligophagous but *Fraxinus* is a preferred host; beetle population potentially impacted by EAB (Shawn Clark in litt.)

APPENDIX 3. (continued)

Taxon	Species	Previous Ranking	Current Ranking	*Fraxinus* Specialist?	Taxonomically Valid?	Herbivore?	Native to U.S.?	Comment(s)
Diptera	*Colpodia temeritatis* Felt	High	Not Imperiled	Misattributed *Fraxinus* Specialist	Invalid Taxonomy	Not an Herbivore		Valid name: *Porricondyla temeritatis*; mycophagous (Raymond Gagné in litt.)
Diptera	*Continaria canadensis* Felt	High	Not Imperiled	Misattributed *Fraxinus* Specialist	Invalid Taxonomy			Valid name: *Contarinia canadensis*; contamination record (Raymond Gagné, 1989; in litt.)
Diptera	*Dasineura* spp.	High	High		Invalid Taxonomy			Record from Solomon et al. (1993) refers to *D. apicata*; (Raymond Gagné in litt.).
Diptera	*Lasioptera fraxiniflora* Felt	High	Not Imperiled	Misattributed *Fraxinus* Specialist	Invalid Taxonomy			Valid name: *Neolasioptera fraxinifolia*; generalist, contaminant (Raymond Gagné, 1989; in litt.)
Diptera	*Lestodiplosis fraxinifolia* Felt	High	Not Imperiled	Misattributed *Fraxinus* Specialist		Not an Herbivore		Generalist predator (Raymond Gagné in litt.)
Diptera	*Rhizomyia fraxinifolia* Felt	High	Not Imperiled	Misattributed *Fraxinus* Specialist				"Very definitely a generalist phyto- or saprophage" (Raymond Gagné in litt.)
Hemiptera	*Anormenis septentrionalis* (Spinola)	Moderate	Not Imperiled	Misattributed *Fraxinus* Specialist				"Feeds on a wide variety of woody and herbaceous plants" (Wilson and McPherson, 1981)
Hemiptera	*Leptoypha elliptica* McAtee	High-Moderate	Not Imperiled	Misattributed *Fraxinus* Specialist				Hosts are *Forestiera ligustrina* and *F. acuminata* (Wheeler, 2002)
Hemiptera	*Leptoypha ilicis* Drake	High to Moderate	Not Imperiled	Misattributed *Fraxinus* Specialist				Hosts are *Forestiera ligustrina* and *F. acuminata* (Wheeler, 2002)
Hemiptera	*Leptoypha mcateei* Drake	Moderate	Not Imperiled	Misattributed *Fraxinus* Specialist				Hosts are *Osmanthus* and *Lyonia* (Drake and Ruhoff, 1965)

APPENDIX 3. (continued)

Taxon	Species	Previous Ranking	Current Ranking	*Fraxinus* Specialist?	Taxonomically Valid?	Herbivore?	Native to U.S.?	Comment(s)
Hemiptera	*Leptoypha nubilis* Drake	High to Moderate	High to Moderate		Invalid Taxonomy			Valid name: *Leptoypha minor*
Hemiptera	*Psyllopsis fraxinicola* Forst.	High	Not Imperiled				Exotic/Introduced Species	European species (Hodkinson, 1988)
Hemiptera	*Thysanocnemis bischoffi* Blatchley	High	High to Moderate		Invalid Taxonomy			Valid name: *Lignyodes bischoffi*
Hemiptera	*Thysanocnemis helvola* LeConte	High	High to Moderate		Invalid Taxonomy			Valid name: *Lignyodes helvolus*
Hymenoptera	*Eurytoma* spp.	High	Not Imperiled	Misattributed *Fraxinus* Specialist		Not an Herbivore		Wasps bulk reared & only associated with ash; species likely parasitoid (Keen, 1958; David Smith in litt.)
Hymenoptera	*Tethida cordigera* (Beauvois)	High	High		Invalid Taxonomy			Valid name: *Tethida barda*
Lepidoptera	*Manduca brontes* (Drury)	High to Moderate	Not Imperiled				Extralimital Species	Neotropical species recorded from Jamaica (Tuttle, 2007)
Lepidoptera	*Podosesia fraxini* (Lugger)	Moderate	Not Imperiled	Misattributed *Fraxinus* Specialist	Invalid Taxonomy			Color form of *Podesia syringa;* oligophagous (Eichlin and Duckworth, 1988)

CHAPTER 3: HOST RANGE AND HOST RESISTANCE

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INTRODUCTION

Since its discovery in North America in 2002, emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB) has infested and killed many millions of ash trees (*Fraxinus* spp.) in forests, woodlots, urban forests, landscapes, and nurseries (Herms and McCullough, 2014). Ash species indigenous to eastern Asia share a coevolutionary history with EAB and are more resistant than evolutionarily naïve hosts indigenous to North America and Europe, presumably because they possess defenses targeted against EAB that have developed through natural selection (Wei et al., 2004, 2007; Liu et al., 2007; Rebek et al., 2008). By 2010, EAB had killed more than 99% of white (*F. americana* L.), green (*F. pennsylvanica* Marsh.), and black (*F. nigra* Marsh.) ash in forests in southeast Michigan near the epicenter of the invasion, and seed production and new seedling germination had ceased (Kashian and Witter, 2011; Klooster et al., 2014). In the Moscow region of Russia, EAB is causing widespread mortality of European ash (*F. excelsior* L.) (Orlova-Bienkowskaja, 2014), which also lacks a coevolutionary history with EAB. While buprestid wood-borers generally colonize only severely weakened or freshly killed trees (Evans et al. 2007), EAB is also killing healthy trees (Cappaert et al., 2005), making the invasions of North America and Russia especially devastating.

HOST RANGE OF EMERALD ASH BORER

Ash species indigenous to east Asia reported as hosts of EAB include Manchurian ash (*F. mandshurica* Rubr.) and two species of Chinese ash (*F. chinensis* Roxb. and *F. rhyncophylla* [Hance] A.E. Murray) (Wei et al., 2004, 2007) (of which the latter is sometimes

given as a subspecies of *F. chinensis*) (Wallander, 2001). These species are presumed to share a coevolutionary history with EAB (Liu et al., 2003, 2007).

In addition to white, green, and black ash, other species indigenous to North America that have been documented as hosts of EAB include pumpkin ash (*F. profunda* [Bush] Bush) (Knight et al., 2013), and blue ash (*F. quadrangulata* Michx.) (Anulewicz et al., 2008; Tanis and McCullough, 2012). Oregon ash (*F. latifolia* Benth.), native to the west coast of North America, was found to be highly susceptible in a common garden study conducted in southeast Michigan (Table 1). Velvet ash (*F. velutina* Torr.), native to the southwest United States and Mexico, has been colonized and killed by EAB when planted as an ornamental in China (Liu et al., 2003; Wang et al., 2010), while freshly cut logs of Shamel (evergreen) ash (*F. uhdei* [Wenz.] Lingl.), which is indigenous to Mexico, were suitable for rearing emerald ash borer larvae in experimental studies (Duan et al., 2013). The susceptibility of Oregon, velvet, and Shamel ash suggests that the southwest and west coast of North America are vulnerable to EAB invasion, depending on the environmental tolerance of the insect.

Some ash species and cultivars that are indigenous to Europe also have been confirmed as hosts for EAB (i.e., supporting successful larval development and adult emergence). Following this borer's introduction to the Moscow region of Russia (Baranchikov et al., 2008), EAB caused widespread mortality of European ash (Orlova-Bienkowskaja, 2014). In a common garden study in southeast Michigan, flowering ash (*F. ornus* L.), Raywood ash (*F. angustifolia* subsp. *oxycarpa* [M. Bieb. ex Willd.] Franco & Rocha Afonso [syn. *F. oxycarpa* M. Bieb. ex Willd.] cv. 'Raywood'), and the European ash cultivar 'Aureafolia' were readily colonized by EAB (Table 1).

Table 1. Survival and canopy condition of ash species and cultivars in 2009, 2011, and 2014 in a common garden established in southeast Michigan in 2004. Canopies of living trees were rated on a scale of 1 (severe decline) to 5 (no decline). Each taxon was replicated 20 times in a randomized complete block design.

Species	Cultivar	Geographic Origin	2009		2011		2014	
			% Survival	Canopy Rating	% Survival	Canopy Rating	% Survival	Canopy Rating
F. mandshurica	seedling origin	Asia	85	4.6	80	4.80	80	4.20
F. nigra x *mandshurica*	Northern Treasure	Asia x North America hybrid	90	4.4	80	4.80	80	4.00
F. excelsior	Aureafolia	Europe	25	3.8	10	5.00	5	2.00
F. ornus	seedling origin	Europe	20	1.5	0	NA	0	0.00
F. angustifolia subsp. *oxycarpa*	Raywood	Europe	35	4.8	30	3.60	0	NA
F. americana	Autumn Applause	North America	40	4.4	25	4.60	20	2.00
F. americana	Autumn Purple	North America	50	4.6	50	4.60	40	2.10
F. americana	seedling origin	North America	85	4.8	70	4.10	45	2.90
F. americana	Sparticus	North America	55	4.9	45	4.90	45	2.10
F. latifolia	seedling origin	North America	25	2.3	5	2.00	0	NA
F. nigra	Fallgold	North America	35	4.5	15	2.70	5	3.00
F. nigra	seedling origin	North America	10	5.0	5	5.00	0	NA
F. pennsylvanica	Cimmaron	North America	40	5.0	40	4.90	35	2.90
F. pennsylvanica	Patmore	North America	30	5.0	30	4.40	15	2.30
F. pennsylvanica	Summit	North America	20	20.0	15	3.30	0	NA
F. quadrangulata	seedling origin	North America	90	4.8	80	4.60	65	2.20

In Japan, species of *Juglans* (walnuts and butternuts), *Ulmus* (elms), and *Pterocarya* (wingnuts) have been reported as hosts for EAB (Haack et al., 2002). However, EAB has not been well studied in Japan, and host records for wood-borers can be unreliable, potentially including species from which adults were collected even when they do not colonize that species in the larval stage, or they may represent taxonomic errors or confusion (e.g. synonymy of separate species) (Muilenburg and Herms, 2012; Haack, 2013). In experimental host range studies, EAB larvae were not able to complete development on American elm (*Ulmus americana* L.), black walnut (*Juglans nigra* L.), hackberry (*Celtis occidentalis* L.), shagbark hickory (*Carya ovata* [Mill.] K. Koch), or on members of the ash family (Oleaceae) tested, including

Japanese tree lilac (*Syringa reticulata* Bl.), swamp privet (*Forestiera acuminata* [Michx.] Poir.), Chinese privet (*Ligustrum sinense* Lour.), and glossy privet (*Ligustrum lucidum* Ait.) (Anulewicz et al., 2006, 2007). Recently, white fringe tree (*Chionanthus virginicus* L.), which is also in the ash family, was confirmed as a larval host for EAB (Cipollini, 2015).

INTERSPECIFIC PATTERNS OF ASH RESISTANCE TO EAB

Emerald ash borer is only occasionally a damaging pest of ash species native to eastern Asia, but has caused widespread mortality of North American ash species planted in China (Wei et al., 2004, 2007). For example, EAB killed all white ash trees planted in

the city of Shenyang, as well as all trees in a 10-year-old white ash planting in the experimental forest of Northeast Forestry University in Harbin. The outbreaks occurred at the same time EAB populations were low on Manchurian ash in neighboring forests. In another study, Liu et al. (2007) observed that green ash was colonized at a higher rate than native Asian species planted at the same site. They concluded based on their field surveys that EAB does not pose a serious threat in China to indigenous ash species. However, Liu et al. (2003) and Wei et al. (2004, 2007) reported that white ash is no longer planted in China and plantings of green ash remain only in localized areas because of past EAB attack. The EAB invasion of Russia west of the Urals has made it apparent that European ash is also highly susceptible to EAB (Orlova-Bienkowskaja, 2014).

Colonization of Asian ash species by EAB has been consistently associated with stressed and dying trees (Wei et al. 2004, 2007; Liu et al. 2007), which suggests that they may be inherently resistant and that EAB has evolved as a secondary colonizer of stressed trees, as is the case with many species of Buprestidae (Evans et al. 2007). Experimental studies of EAB adult host preference and larval performance are consistent with this hypothesis. For example, EAB adults preferred to feed upon foliage from leaves from trees stressed by girdling (Chen and Poland, 2009), injured by adult feeding damage, or induced by methyl jasmonate (Rodriguez-Saona et al., 2006). In field experiments, adult-landing rates were higher on girdled trees, as were larval densities and growth rates (McCullough et al., 2009; Tluczek et al., 2011). Jennings et al. (2014) found that females preferred to oviposit on declining trees that were previously infested by EAB rather than on healthy trees.

All North American ash species encountered to date by EAB have proven susceptible to varying degrees (Herms and McCullough, 2014). Black, green, and white ash are highly susceptible (Klooster et al., 2014), although white ash is somewhat less preferred, possibly because its smoother bark (at least in younger trees) may be a less preferred oviposition substrate (Anulewicz et al., 2008). In forests, trees with rougher bark were reported to be killed at a slightly faster rate than smoother barked

trees (Marshall et al., 2013). However, at the stand level, black, white, and green ash declined at similar rates, with populations of all three species ultimately reaching greater than 99% mortality at about the same time (Smith, 2006; Smith et al., 2015; Klooster et al., 2014). Blue ash (*F. quadrangulata*) appears to be the least vulnerable North American ash species encountered by EAB to date. Tanis and McCullough (2012) observed that more than 60% of blue ash in wooded areas in southeastern Michigan appeared healthy, while white ash with trunks greater than 10 cm in diameter were all killed.

Patterns of ash decline and mortality in an ongoing common garden study established at Michigan State University's Tollgate Education Center in Novi, Michigan in 2004 are largely consistent with the hypothesis that coevolved species indigenous to Asia are more resistant than evolutionary naïve hosts native to North America and Europe (Table 1). The resident EAB population was low when the plot was established as most trees in the region had been killed. As EAB populations began to resurge and susceptible trees in the plot began to be killed, Manchurian ash had the highest rate of survival and little canopy decline. Mortality of Manchurian ash that did occur was concentrated in the first few years after planting, perhaps due to transplant stress. The only tree killed after 2009 had its trunk badly injured by a deer rub. The high EAB resistance of this Manchurian ash population of seedling origin is consistent with that observed by Rebek et al. (2008) for the clonal Manchurian ash cultivar 'Mancana,' suggesting that EAB resistance is a species-level trait.

Fraxinus x 'Northern Treasure' ash, which is a Manchurian (Asian) x black ash (North American) hybrid (Davidson, 1999) had similarly high survival and low canopy decline, suggesting introgression of Manchurian ash resistance genes into the hybrid (Table 1). However, this pattern contrasts sharply with that observed by Rebek et al. (2008), who found 'Northern Treasure' ash to be highly susceptible to EAB. This suggests there is taxonomic confusion in the nursery industry surrounding this cultivar that has yet to be resolved.

Most North American species and cultivars in the common garden study experienced substantial

mortality, with green ash cultivars, black ash, and Oregon ash declining more rapidly than white ash cultivars (Table 1). Blue ash has survived at a higher rate than other North American species, but by 2014 had lower survival and greater canopy decline than Manchurian ash. Furthermore, decline and mortality of blue ash increased over time, suggesting that surviving trees may continue to succumb to EAB as other hosts are eliminated. The European species and cultivars evaluated in the common garden also experienced high decline and mortality, including *F. ornus*, *F. excelsior* 'Aureafolia', and *F. angustifolia* subsp. *oxycarpa* 'Raywood' (Table 1).

The high level of resistance of Asian ash relative to North American and European species has been attributed to a coevolutionary history that has stabilized the interaction between EAB and its indigenous hosts in Asia (Liu et al., 2003, 2007; Rebek et al., 2008). Conversely, the widespread mortality of Nearctic and European ash species in invaded regions has been attributed to very high susceptibility of evolutionarily naïve host plants, which has facilitated the spread and population growth of EAB in defense-free space (Gandhi and Herms, 2010; Raupp et al., 2010). A similar pattern has been observed for birch (*Betula* spp.) resistance to congeneric bronze birch borer (*Agrilus anxius* Gory), which is endemic to North America. North American birch (*Betula*) species share a coevolutionary history with bronze birch borer and are much more resistant than evolutionarily naïve Eurasian birch species (Nielsen et al., 2011).

MECHANISMS OF RESISTANCE OF ASH TO EAB

Host plant resistance to insects is considered a continuous trait ranging from complete immunity at one end of the spectrum to extreme susceptibility at the other (Painter, 1958; Beck, 1965). Mechanisms of resistance have been broadly classified as antibiosis (plants traits that lower herbivore performance, including fecundity, growth, and survival), antixenosis (plant traits that reduce behavioral preferences for feeding or oviposition), and tolerance (traits that allow a plant to grow or repair injury to

a greater degree than another host experiencing the same amount of herbivory) (Painter, 1958; Beck, 1965; Wiseman, 1985). Biogeographically, resistance has been classified as coevolved (host defenses resulting from natural selection) or allopatric (herbivore lacks the pre-adaptions needed to perform well on a novel host) (Harris, 1975).

Because the devastating impact of EAB on its host is due to larval feeding, research on mechanisms of resistance have focused on factors affecting larval density and survival, including traits affecting female fecundity and oviposition preferences, as well as stem defenses and nutritional quality that influence larval establishment, growth, and survival. However, the relative importance of antibiosis and antixenosis in inter- or intraspecific variation in resistance of ash to EAB has yet to be fully delineated, and the role of tolerance has not been investigated.

EAB adults have demonstrated variation in host preference for maturation feeding and oviposition. Pureswaran and Poland (2009) found that adults preferred to feed on green, black, and white ash relative to Manchurian, blue, and European ash. This pattern corresponds largely with patterns of resistance and vulnerability observed in the field, with the most susceptible species also being preferred (with the exception of European ash, which is highly susceptible), and suggests that there might be general correspondence between adult feeding preferences and female oviposition preferences. Consistent with this hypothesis, Rigsby et al. (2014) observed in two common garden experiments that females oviposited much more extensively on white and green ash than on Manchurian ash, and Anulewicz et al. (2008) found that females preferred to oviposit on green and white ash relative to blue ash. These findings also suggest that oviposition preference is an important determinant of interspecific variation in ash mortality and decline observed in field studies.

Mechanisms of tree resistance to wood-borer larval feeding are not well understood but have been postulated to result from integrated constitutive and induced physical and chemical defenses of the phloem and outer xylem (Matson and Hain, 1985; Dunn et al., 1990; Muilenburg and Herms, 2012). Studies to elucidate the mechanism of resistance of Manchurian

ash to EAB have focused on comparing its induced and constitutive phloem chemistry to that of susceptible species. Eyles et al. (2007) compared the constitutive phloem phenolic chemistry of dormant stems of Manchurian, white, and green ash and identified compounds present in the Manchurian ash cultivar 'Mancana' that were not present in the more susceptible species, including several hydroxycoumarins and two phenylethanoids (calceolariosides A and B) and suggested they might represent potential EAB resistance mechanisms. In a similar analysis conducted during the growing season, Cipollini et al. (2011) also found the constitutive phenolic profile of Manchurian to be distinctly different from that of green and white ash, observing patterns of qualitative variation similar to those reported by Eyles et al. (2007).

In more phylogenetically controlled comparisons, however, Whitehill et al. (2012) detected these putative resistance compounds in concentrations comparable to or higher in highly susceptible black and European ash, which are much more closely related to Manchurian ash than are green and white ash. This strongly suggests that hydroxycoumarins and calceolariosides A and B are, in fact, not responsible for the high resistance of Manchurian ash. Pinoresinol dihexoside and a tentatively identified coumarin derivative were the only phenolic compounds detected that were unique to Manchurian ash, which suggests that the other 25 phenolic compounds detected are unlikely to play a role in resistance unless they synergize other classes of compounds that are unique to Manchurian ash (Whitehill et al., 2012). They did speculate that that two unique lignans may serve as markers for, or contribute directly to, the higher EAB resistance of Manchurian ash (Whitehill et al., 2012). They also proposed that the very distinct phenolic profile of blue ash may contribute to its higher level of resistance to EAB relative to green and white ash.

The constitutive protein chemistry of ash phloem also has been examined. Manchurian ash had higher soluble protein concentration and a higher rate of browning (oxidation) reaction than did green or white ash, although trypsin inhibitor activity, peroxidase activity, and total soluble phenolic concentrations of Manchurian ash were lower than in at least one of the more susceptible species (Cipollini et al.,

2011). Whitehill et al. (2011) compared the phloem proteomes of Manchurian, black, green, and white ash, and they identified several proteins implicated as defenses in other species that were constitutively over-expressed in Manchurian ash relative to the other species and might contribute to resistance. These include a PR-10 protein, phenylcoumarin benzylic ether reductase, an aspartic protease, and ascorbate peroxidase.

Nutritional quality of plants also contributes to variation in their resistance to herbivores, and Hill et al. (2012) quantified phloem compounds in Manchurian, green, and white ash that are thought to be of nutritional significance to EAB larvae, including nitrogen, total protein, free amino acids, total soluble sugars, and macro- and micro-nutrients. They found few differences, although concentrations of the amino acid proline, as well as the amino acid derivatives tyramine and tyrosol were higher in Manchurian ash. Chen et al. (2011) reported that larval growth was reduced on artificial diets in which protein or amino acids were limiting, and that the downward orientation of feeding as larvae formed their galleries allowed them to consume phloem with higher water and essential amino acid concentrations.

Chakraborty et al. (2014) examined induced responses of Manchurian and black ash phloem to larval feeding, which has received far less attention than constitutive patterns. They found that EAB larval biomass was lower on Manchurian ash, which provides evidence that antibiosis as well as ovipositional non-preference contributes to high resistance of Manchurian ash, as does the observation of Duan et al. (2012) that host plant factors caused higher mortality of larvae feeding on Asian species of ash than on North American green ash. EAB larval feeding induced higher concentrations of pinoresinal A in Manchurian than black ash, which Chakraborty et al. (2014) speculated might contribute to resistance. Drought stress increased larval performance on both species, which is consistent with the hypothesis that stress increases host quality.

Counter adaptations of EAB larvae to ash defenses have also been examined. Transcriptomic studies of EAB have focused on larval enzymes that function in detoxification of host defenses (Rajarapu et al., 2011;

Rajarapu and Mittapalli, 2013). Chen et al. (2012) found that phenolic concentrations were lower in EAB frass than in phloem tissue and inferred that larvae may excrete phenolics and/or convert them to non-phenolic compounds before excretion.

BREEDING FOR RESISTANCE TO EAB

Because of their inherent resistance to EAB, Asian ash species are a likely source of resistance genes that might be introgressed into North American species (Whitehill et al., 2011), and efforts to breed EAB-resistant ash are ongoing (Koch et al., 2012). Extensive surveys of ash stands in Michigan and Ohio have revealed a very small proportion of ash that remain healthy where EAB-ash induced mortality exceeds 99%, and thus may provide a potential source of allopatric resistance genes in native ash populations (Knight et al., 2012). However, it remains to be documented whether these genotypes are truly resistant or just lucky. Genomic sequencing of Asian and North American ash species have also been conducted to provide a molecular foundation for targeted breeding (Bai et al., 2011; Rivera-Vega et al., 2012) that ultimately may lead to restoration of ash to urban and natural forests of North America.

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CHAPTER 4: OTHER OPTIONS FOR EMERALD ASH BORER MANAGEMENT: ERADICATION AND CHEMICAL CONTROL

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THE EARLY YEARS — COULD EAB HAVE BEEN CONTAINED OR ERADICATED?

Following identification of emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB) (Coleop.: Buprestidae) in Michigan in July 2002, state and federal regulatory officials began surveys to delimit the infestated area in the greater Detroit area (Cappaert et al., 2005; Herms and McCullough, 2014). It rapidly became clear that EAB was causing substantial ash mortality and decline, but there was little information available on even basic aspects of EAB biology. No species of *Agrilus* beetles were known to produce long range sex or aggregation pheromones, and there were certainly no traps or lures available for EAB surveys. A high proportion of ash in the suburban municipalities northwest of Detroit was dead or dying, so regulatory personnel conducted visual surveys along transects radiating out from the known infestation. Survey crews checked ash trees for symptoms such as canopy dieback, bark cracks (revealing EAB larval galleries), and epicormic sprouts on large branches or the trunks of infested trees. By autumn 2003, six counties in southeast Michigan were quarantined and at the time, regulatory officials believed the quarantine boundaries extended well beyond the actual infestation (Cappaert et al., 2005; Poland and McCullough, 2006; Siegert et al., 2014).

Along with visual surveys, officials in Michigan initiated trace-backs and trace forwards in 2002 and 2003 to track ash nursery trees shipped from the infested area. Ash, primarily cultivars of green ash (*Fraxinus pennsylvanica* Marshall) and white ash (*F. americana* L.) were abundant in most commercial nurseries in southeast Michigan when EAB was identified. Ash species were popular because they

tolerate the often stressful conditions found in urban environments (Schoon, 1993; MacFarlane and Meyer, 2005; Poland and McCullough, 2006) and were commonly planted in commercial landscapes, along roads and highways, as well as on private residential property. Ash trees near sawmills that processed ash logs, along with ash in campgrounds and tourist destinations likely to attract people with firewood, were also intensively surveyed.

Eradication of EAB within the six counties in southeast Michigan was never considered a realistic option, given the millions of ash trees in urban, residential, and forested areas and the geographic extent of the infestation. Landscapers had begun treating some declining ash trees in the Detroit area with insecticides even before EAB was identified in 2002. Treatment efficacy varied but none provided 100% control (McCullough et al., 2005, 2006; Herms et al. 2014). Moreover, there were no practical or economically feasible means to treat the millions of ash trees growing in the affected areas (Cappaert et al., 2005; Herms and McCullough, 2014).

It was clear, however, that continued spread of EAB would threaten more than 8 billion ash in U.S. forests along with millions of ash in urban landscapes. A strategy similar to that applied to large wildfires was proposed by scientists and regulatory officials appointed to the EAB Science Advisory Panel. Nearly complete mortality of ash within the infested area, which was already underway, would eventually lead to a substantial drop in EAB density in the core of the infestation and over time, the severely affected area would expand. If the advancing front of the infestation could be contained or at least slowed below the rate at which the core expanded, the EAB population would presumably collapse as fewer and

fewer host trees became available. Regulations to restrict transport of ash trees, logs, firewood, and related materials out of the quarantined area were part of the effort to contain the infestation (Federal Register, 2003; Herms and McCullough, 2014).

Officials initially considered removing ash trees in a 5-10 km band around the infested area to deplete potential hosts for dispersing EAB adults. This strategy, referred to as a firebreak or an ash-free zone (Herms and McCullough, 2014), was never attempted in the United States. As surveys continued, the extent of the main EAB infestation in southeast Michigan and northwest Ohio became apparent. Officials realized that the inability to accurately delineate the infestation, the logistical problems of establishing an ash-free band, and the costs of creating such a band were insurmountable. Canadian officials did attempt to establish an ash-free zone in Ontario in 2004, but infested trees were soon found beyond this zone and the firebreak idea was abandoned.

Along with efforts to contain or slow expansion of the main EAB infestation, officials determined that localized "outlier" infestations beyond the quarantine zone would be aggressively treated with the goal of eradication. These satellite populations of EAB, often referred to as "outliers," originated from long distance transport of infested ash nursery trees, logs, or firewood. Large scale field studies that involved systematically felling and debarking ash trees around a known origin showed that while EAB females laid eggs on trees at least 750 m from their emergence point, most eggs were laid within 100 m of the adult beetles' emergence point (Mercader et al., 2009; Siegert et al., 2010). Regulatory officials determined that eradication projects would encompass an area bounded by a perimeter 800 m beyond the furthest ash tree known to be infested. This distance represented a compromise between the need to eliminate infested but non-symptomatic trees and logistical and economic constraints associated with such a substantial undertaking (Herms and McCullough, 2014). Within the eradication project area, every ash tree greater than 2.5 cm in diameter was felled, sectioned and transported to a disposal yard where the material could be chipped. Numerous outlier sites, primarily in Michigan, Ohio, and Indiana,

were targets for eradication between 2003 and 2006 (Cappaert et al., 2005; Herms and McCullough, 2014). The most extensive EAB eradication project occurred in Maryland, where more than 42,000 ash trees were removed between 2003 and 2009 across an area that eventually encompassed nearly 70 km^2 (MD-DNR, 2014).

Eradication efforts, with the exception of Maryland, were abandoned in 2006, in part because funds for eradication, surveys, and related activities were decreasing, but also because outlier populations of EAB continued to be found well beyond the quarantine boundaries (GAO, 2006; Herms and McCullough, 2014). Between 2004-2006, state regulatory officials in Michigan, Indiana and Ohio established grids of small ($\approx$ 15 cm diameter) ash detection trees, typically in right-of-ways along highways and roads (Rauscher, 2006; Hunt, 2007). These trees were girdled in spring, making them highly attractive to adult EAB during the summer, then were debarked in autumn or winter to determine if larvae were present (McCullough et al., 2009). Using girdled trees, along with increased public awareness of EAB, led to the identification of several previously unknown EAB infestations.

As scientists learned more about EAB, it became clear that visual surveys to identify infested trees for detection or to delineate an infestation were inadequate. External evidence of EAB infestation is not apparent until larval densities reach moderate or high levels, while recently infested trees with low larval densities exhibit few, if any, symptoms (Poland and McCullough, 2006; Poland et al., 2011). Moreover, in relatively healthy trees, most EAB larvae require two years to complete development (Siegert et al., 2010; Tluczek et al., 2011). Therefore, trees are usually infested for at least 3-4 years before any external symptoms become apparent. More recent evidence also suggests a small proportion of mature, mated females likely disperse relatively long distances, despite an abundance of suitable host trees in the local vicinity (McCullough et al., 2011a; Mercader et al., 2012), contributing to the difficulty of delineating or detecting new infestations. Eradication activities undoubtedly eliminated a very high proportion of infested trees and developing larvae, but it remains unclear as to whether any projects were successful. Infestations near

eradication areas could represent reproduction by EAB that had already dispersed beyond the boundaries of a project area or may reflect subsequent expansion or immigration of beetles from other populations (Herms and McCullough, 2014).

SYSTEMIC INSECTICIDES, EAB, AND ASH TREES

Once eradication efforts ceased, landowners and residents were left to deal with ash trees and EAB on their own. Early studies soon after EAB was identified in North America showed spraying the foliage and upper canopy of landscape trees with relatively persistent insecticides (e.g., bifenthrin, cyfluthrin) could effectively control adult EAB and protect trees (McCullough et al., 2005, Herms et al., 2014). Sprays were not popular, however, because of problems such as drift and possible environmental contamination, potential effects on non-target organisms such as pollinators and beneficial predatory insects, and possible applicator exposure. In addition, adequate coverage of the upper canopy of large trees was difficult and trees in many locations could not be reached with spray equipment.

Fortunately, options for protecting landscape ash trees in urban areas from EAB have progressed substantially in the past decade. Systemic insecticides are now used to treat the vast majority of ash trees in urban areas where EAB is present. These products are applied by injecting the insecticide into the outer sapwood (xylem) around the base of the trunk of the tree (e.g., emamectin benzoate, imidacloprid, azadiractin) or applying it to the soil around the base of the tree for uptake by roots (e.g., dinotefuran, imidacloprid) (Herms et al., 2014). Products with dinotefuran, a highly soluble compound, can also be applied as a basal trunk spray (McCullough et al., 2011b; Herms et al., 2014). Trees transport the insecticide in xylem tissue from the base of the tree up to branches and foliage in the canopy (Sur and Stork, 2003; Mota-Sanchez et al., 2009; Tanis et al., 2012). Systemic products eliminate most problems associated with cover sprays of insecticides. They must be applied, however, before high densities of EAB larvae injure the vascular system of an ash tree (Herms et al., 2014).

When EAB was first identified, only a few systemic insecticide products were available. Imidacloprid was the active ingredient in most of those products and was applied either as a soil drench or by injecting the product into the base of the tree. Field trials with these products, however, yielded inconsistent results (McCullough et al., 2006; Herms et al., 2014). Products protected trees from EAB injury in some sites, but in other sites, the same treatments were not effective. In some studies, EAB damage continued to increase, despite annual insecticide applications. Treated trees sometimes lived a few years longer than untreated trees, but still succumbed to EAB, despite the investment in treatment (McCullough et al., 2006).

Research continued and new systemic insecticides became available, application technology improved, and our understanding of how to optimize these treatments advanced considerably. A product with the active ingredient emamectin benzoate, first registered in the United States in 2010, is the most effective systemic insecticide currently available for EAB control (Smitley et al., 2010; McCullough et al., 2011b; Herms et al., 2014). Many field trials showed that emamectin benzoate consistently provided 2-3 years of nearly complete EAB control, even when local EAB densities were high (Smitley et al., 2010; McCullough et al., 2011b; Herms et al., 2014). Many cities in the United States, as well as private landowners, are now protecting valuable ash trees from EAB with this product. Economic analyses showed costs of protecting landscape trees with the emamectin benzoate product to be substantially lower over time than the costs of removing trees killed by EAB (McCullough and Mercader, 2012; Van Atta et al., 2012). Treating a portion of the trees may also slow the rate of EAB population growth in a localized area (Mercader et al., 2011a,b; McCullough and Mercader, 2012). Insecticides with azadirachtin, a compound derived from the neem tree (*Azadirachta indica* A. Juss.) as the active ingredient, have recently become available for EAB control (McKenzie et al., 2010; Herms et al., 2014) in the United States and Canada and provide 1-2 years of protection, depending on local EAB density. Unlike other insecticides, which are toxic to EAB adults and larvae,

azadirachtin products affect EAB reproduction and development of young larvae (McKenzie et al., 2010; Kreutzweiser et al. 2011, DGM, unpubl. data). Basal trunk sprays of dinotefuran, a highly soluble "new generation" neonicointoid product, are effective if applied annually and are popular among arborists, especially when many small trees require treatment (McCullough et al., 2011b; Herms et al., 2014). Dinotefuran sprayed on the trunk moves through the outer bark and into the xylem, where it is then transported to the canopy. Imidacloprid insecticides, which also must be applied annually, continue to be used for EAB control, although effectiveness of these products varies considerably (Herms et al., 2014).

INTEGRATING SYSTEMIC INSECTICIDES AND BIOLOGICAL CONTROL

It is important to note that biological control and systemic insecticides are not mutually exclusive and in combination may yield additive or even synergistic (e.g., superadditive) effects on EAB population growth (Barclay and Li, 1991; Berec et al., 2007; Suckling et al., 2012). Simulations have shown that decreasing pest density with a density-independent tactic such as systemic insecticides may enhance the effectiveness of density-dependent tactics, including biological control, particularly if a parasitoid displays nonrandom searching behavior (Barclay and Li, 1991; Suckling et al., 2012). Larval parasitoids and woodpeckers will not attack dead EAB larvae. Thus, unlike cover sprays, which are likely to affect a wide range of natural enemies, systemic products should have negligible effects on populations of native or introduced parasitoids (or predators) of EAB. Minimally, an additive effect should occur because systemic insecticides and biological control agents target different life stages of EAB. Systemic insecticides affect adult beetles as they feed on leaves and control newly hatched, neonate larvae, whereas native and introduced EAB parasitoids attack EAB eggs or late instar larvae. A synergistic effect may occur if untreated trees near trees treated with the emamectin benzoate product benefit from lower local EAB populations (McCullough and Mercader, 2012). Such trees would

provide a consistent, multi-year source of EAB eggs and larvae to retain and support parasitoids. A similar interaction may occur in areas where the native ash species demonstrate some level of resistance to EAB. Black ash (*Fraxinus nigra* Marshall) and green ash, for example, are highly attractive and vulnerable hosts for EAB, white ash is intermediate, but healthy blue ash (*Fraxinus quadrangulata* Michx.) trees do exhibit resistance (Cappaert et al., 2005; Anulewicz et al., 2007; Limback, 2010; Tanis and McCullough, 2012). Interactions between parasitoids and EAB in areas where a portion of ash trees are protected with effective systemic insecticides or in sites with relatively resistant ash species remain to be determined.

FEWER OPTIONS TO PROTECT ASH TREES IN FORESTS

While landscape trees can now be effectively treated with systemic insecticides, these products are not practical options for ash trees in forests, woodlots, riparian zones, or other natural areas. In part, this reflects the substantially higher economic value of individual urban landscape trees compared to forest trees. Many practical and environmental concerns also limit the use of chemical insecticides, including systemic products, in forests and natural areas.

Options for managing EAB in forests may someday include microbial insecticides or perhaps entomopathogenic products. In the United States and many other countries, populations of foliar feeding Lepidoptera are commonly suppressed (or even eradicated) with aerial applications of Bt products (*Bacillus thuringiensis* var. *kurstaki*) (Tobin and Blackburn, 2007; Hajek and Tobin, 2011; Suckling et al., 2012; Tobin et al., 2014). However, there are as yet no effective microbial insecticides for EAB that can be applied in forested settings or across large areas. Researchers from federal agencies, along with private companies, continue to investigate Bt strains that could potentially be used to control adult EAB beetles (Bauer et al., 2012). Aerial applications of Spinosad (*Saccharopolyspora spinosa* Mertz and Yao) in woodlots with EAB infestations have been evaluated on a trial basis (Lewis and Smitley, 2012). Spinosad is a microbial

product that affects a broad range of plant-feeding insects, but may be less likely to harm predatory insects or parasitoids. Other research has focused on entomopathogens, such as the generalist fungal pathogen *Beauveria bassiana* (Bals.-Criv.) Vuill.*,* and efficient methods to infect EAB or enhance efficacy or persistence of these products (Liu and Bauer, 2006; Lyons et al., 2012).

Bioinsecticides could eventually play a role in integrated management programs for EAB in forests, if they can be economically produced, effectively applied, and can suppress EAB without harming a wide range of other invertebrates. Life history traits, however, suggest EAB may be less amenable to control via aerial application of Bt or related products than Lepidopteran forest pests like gypsy moth (*Lymantria dispar* L). Individual EAB adults have a relatively long life span and in a given area, adult EAB may be active for at least 10 to 12 weeks during the summer. In addition, EAB adults, particularly mature females, spend considerable time on branches or the trunk. Aerially applied products will likely, therefore, need to penetrate the canopy of overstory trees, which can be challenging during the summer. Whether aerially applied microbial insecticides can persist long enough and reach foliage where most adult EAB are feeding under operational conditions remains to be determined. Given these problems, effective biological control of EAB, whether by native natural enemies or introduced parasitoids, may be critical for preventing the functional loss of many ash species in forest ecosystems across North America (Burr and McCullough, 2014; Klooster et al., 2014; Knight et al., 2013).

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CHAPTER 5: HISTORY OF EMERALD ASH BORER BIOLOGICAL CONTROL

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INTRODUCTION

The search for natural enemies of the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), in northeastern Asia, its native range, was initiated within a year of its discovery in the United States (Bauer et al., 2005, 2014). Although the official response to EAB's invasion in both the United States and Canada was to regulate and attempt to eradicate EAB, the size and complexity of the outbreak prompted scientists and policy makers to support exploration for natural enemies as a potential tool for management of EAB. In 2008, when populations of EAB were already known to occur in nine states, the U.S. government moved from a policy of eradication to one of management (USDA-APHIS, 2013). By this time, scientists had completed all the steps necessary to secure permits for field release of three EAB natural enemies (host range assessment and safety evaluations) from China, and the use of these parasitoids was incorporated into the EAB management plan. This chapter documents the considerable efforts that went into making this possible in just five years.

EAB NATURAL ENEMIES FROM THE UNITED STATES

Before pursuing classical biological control, it is important to determine if locally present natural enemies are attacking and affecting populations of the pest in the invaded area. After the discovery of EAB in Michigan in 2002, studies were conducted in Michigan and Pennsylvania that found several larval parasitoids but no egg parasitoids (Bauer et al. 2004, 2005; Duan et al. 2009, 2013a). Most of these larval parasitoids are associated with native species of *Agrilus* (Taylor et al., 2012). Parasitism rates measured soon after the discovery of EAB in the United States were low (<1% in Michigan and 5% in Pennsylvania) compared to rates seen in Asia (Liu et al., 2003, 2007; Yang et al., 2005; Duan et al., 2012a) and rates reported in the literature for native *Agrilus* spp. in the United States. This low level of natural enemy attack on EAB was clearly inadequate to suppress EAB populations. Entomopathogenic fungi caused about 2% mortality of EAB life stages under the bark (Bauer et al., 2004). Predaceous beetles and woodpeckers also attacked EAB, but not in sufficient numbers to significantly affect EAB densities (Bauer et al., 2004; Lindell et al., 2008; Duan et al., 2011; Jennings et al., 2013). The lack of natural enemies capable of suppressing EAB populations below a density permitting survival of native ash trees was of great importance (Federal Register, 2007), and this risk supported the decision to introduce parasitoids that coevolved with EAB in Asia for biological control of EAB in North America (Bauer et al., 2008).

EXPLORATION IN ASIA FOR EMERALD ASH BORER NATURAL ENEMIES

Natural Enemy Surveys in China

Initially, exploration for EAB natural enemies focused on China (Liu et al., 2003), investigating 11 locations in six areas where EAB had previously been reported: the provinces of Heilongjiang, Jilin, Liaoning, Hebei,

and Shandong, as well as the city-province of Tianjin (Yu, 1992; Xu, 2003). EAB was found in all provinces except Shandong (Liu et al., 2003). By 2002, these surveys in China had identified two larval parasitoids of EAB, *Spathius* sp. (Hymenoptera: Braconidae) and *Tetrastichus* sp. (Hymenoptera: Eulophidae) (Liu et al., 2003), and later in 2004, one egg parasitoid, *Oobius* sp. (Hymeoptera: Encyrtidae) was found (Zhang et al., 2005). These species were later described as *Spathius agrili* Yang (Yang et al., 2005), *Tetrastichus planipennisi* Yang (Yang et al., 2006), and *Oobius agrili* Zhang and Huang (Zhang et al., 2005).

In depth studies of the population dynamics of EAB and its natural enemies were carried out in Jilin and Liaoning Provinces in northeastern China in 2004 and 2005. The two most abundant natural enemies of EAB, collected from EAB-infested *Fraxinus pennsylvanica* Marshall (native to eastern North America) in Jilin Province, were the larval parasitoid *T. planipennisi* and the egg parasitoid *O. agrili* (Liu et al., 2007). During the course of this study, these two parasitoid species reduced EAB densities by an estimated 74% in the infested trees. *Tetrastichus planipennisi* is also known from other provinces in northeast China, including Hebei (LSB unpublished data) and Heilongjiang (Yang et al., 2006).

Tetrastichus planipennisi is a gregarious endoparasitoid that attacks EAB larvae by drilling through the tree bark with its ovipositor. Brood sizes (number of eggs laid per host larva) range from 4 to 172 (Yang et al., 2006; Ulyshen et al., 2010). The EAB larva continues feeding as parasitoid larvae develop inside its body; eventually the parasitoids consume most of the EAB larva and emerge into the gallery, where they pupate and develop into adults. Adult wasps chew through the bark and emerge. There are up to four such generations per year. Average parasitism of larvae by *T. planipennisi* in China was 22.4%, but reached up to 65% (Liu et al., 2003, 2007; Yang et al., 2006).

Oobius agrili is a small (1 mm long), parthenogenic wasp that lays its eggs singly inside EAB eggs. In general, adults from the first generation emerge to attack more EAB eggs, while wasps of the second generation enter diapause and overwinter inside the host egg. However, some individuals of the first generation of *O. agrili* do not emerge until the following spring. Parasitism of EAB eggs was as high as 61.5% by the end of the field season at some locations in northeastern China (Liu et al., 2007).

Although scarce in northeast China, *S. agrili*, the third EAB parasitoid from China, was most abundant in Tianjin City in planted stands of *Fraxinus velutina* Torr. (native to the southwestern North America) (Xu, 2003; Liu et al., 2003; Yang et al., 2005). Parasitism of EAB larvae by *S. agrili* was as high as 90% in some stands in Tianjin by the end of the season (Yang et al., 2010). *Spathius agrili* females use their ovipositor to drill through the bark into the EAB larva, inject venom to paralyze it, and lay an average of five eggs per host larva (Gould et al., 2011). The parasitoid larvae hatch and feed externally on the EAB larva. Mature parasitoids pupate inside cocoons in the gallery, and adult wasps chew through the bark to emerge. Yang et al. (2010) estimate that *S. agrili* completes 3-4 generations per year in Tianjin.

A fourth species of parasitic wasp, in the genus *Sclerodermus* (Hymenoptera: Bethylidae), was discovered parasitizing mature EAB larvae and pupae in Tianjin (Wu et al., 2008) and later described as *Sclerodermus pupariae* Yang et Yao (Yang et al., 2012). After locating a host, female wasps chew through the bark and use their strong front legs to excavate a tunnel through the tightly packed EAB frass in the host gallery to locate host larvae or pupae. They then sting and paralyze their host, feed on the hemolymph, and lay an average of 40 eggs per host. After hatching, *S. pupariae* larvae feed externally on the host and, with some maternal care during the larval stage, pupate inside cocoons. Approximately 13% of the EAB sampled in Tianjin were parasitized by *S. pupariae* (Yang et al., 2012).

Natural Enemy Surveys in the Russian Far East

Exploration for EAB parasitoids in Russia was concentrated near Vladivostok and Khabarovsk, where EAB is native (Williams et al., 2010; Duan et al., 2012a). As in China, ash trees native to Asia (*F. mandshurica* Rupr. and *F. chinesis* Roxb. subsp. *rhynchophylla*) and to North America (*F.

pennsylvanica) were sampled in both natural forests and urban areas. Little parasitism was noted in Khabarovsk, but three larval parasitoid species were recovered in the Vladivostok region (Duan et al., 2012a), as well as a strain of egg parasitoid in the genus *Oobius* that completes one generation per year and, based on DNA evidence, appears to be a different species than *O. agrili* from China (JJD, unpublished data). The larval parasitoids, mainly attacking EAB in *F. pennsylvanica*, included *T. planipennisi, Atanycolus nigriventris* Vojnovskaja-Krieger, and a previously unknown species of *Spathius*. The latter species was recently described as *Spathius galinae* Belokobylskij, and although its general biology is similar to that of *S. agrili,* it has a longer ovipositor and may be better adapted to the cold climate of the north central United States (Belokobylskij et al., 2012). Depending on the site and year, parasitism rates were approximately 24% for *T. planipennisi*, 23% for *A. nigriventris*, 76% for *S. galinae*, and 28% for *O. agrili* (Duan et al. 2012a; JJD unpublished data).

Natural Enemy Surveys in South Korea

Emerald ash borer is quite rare in South Korea and is probably kept in check by a combination of host resistance and natural enemies (Williams et al., 2010). After several years of exploration, two EAB populations were discovered attacking *F. chinesis* subsp. *rhynchophylla* and *F. mandshurica*. One EAB population was found in stressed landscape trees near the city of Daejeon, and the other was on trees damaged during construction further north at a site near Seoul. At these sites, three natural enemy species were discovered: a larval ectoparasitoid later identified as *S. galinae,* a larval endoparasitoid tentatively identified as *Tetrastichus telon* Graham*,* and the clerid beetle *Teneroides maculicollis* Lewis. The clerid attacked EAB in the overwintering pupal chamber, where it pupated after consuming the host. The three species were brought to a United States quarantine facility; however, colonies could not be established.

Surveys for Natural Enemies in Mongolia/Japan

Although *A. planipennis* has been reported from Japan (Schaefer, 2004) it is quite rare (Haack et al., 2002). A Japanese buprestid specialist returned to a locality from which *A. planipennis* had previously been collected and found a single adult beetle on a leaf. In Fukui Prefecture, Honshu, Japan, EAB is listed as endangered because only two collection locations have been recorded. Natural enemies of EAB were not recovered in Japan (Schaefer, 2005).

Foreign exploration in Mongolia was even more fruitless (Schaefer, 2005). Not only were no EAB populations found, but collectors could not even find ash trees. No species in the genus *Fraxinus* occur in the published list of Mongolian vascular plants, and *A. planipennis* has not been recorded from Mongolia. Schaefer (2005) hypothesized that someone may have erred and associated collection of EAB from Mongolia when the discovery may instead have been in Inner Mongolia, China, where EAB is thought to occur.

SELECTION OF POTENTIAL BIOLOGICAL CONTROL AGENTS

Just because a natural enemy is found attacking the target pest in its native range does not necessarily make it suitable for use as a biological control agent (González and Gilstrap, 1992). *Sclerodermus pupariae* has several features lowering its potential as a biological control agent: (1) many females lack wings and would not disperse well, (2) the percentage parasitism observed in China was low, (3) it had a broad host range, and (4) members of the genus are known to sting humans (Gordh and Maczar, 1990; Tang et al., 2012; Yang et al., 2012; Wei et al., 2013). Therefore, this species was not considered for importation as a potential biological control agent for EAB. In contrast, *A. nigriventris* has better potential for use against EAB; however, scientists have yet to succeed in getting them to mate in the laboratory (JJD personal communication). Host specificity testing would also need to be conducted carefully

because *Atanycolus* species native to the United States tend to have a broad host range and are known to attack EAB, often in large numbers (Cappaert and McCullough, 2009).

Oobius agrili, *T. planipennisi*, *S. agrili*, and *S. galinae* were all considered promising candidates for biological control. All four species possess characteristics considered by Kimberling (2004) as enhancing the likelihood of successful biocontrol: female-biased sex ratio or parthenogenesis, a short generation time, and high rates of parasitism and fecundity. These four species were imported into quarantine in the United States for host range testing.

Quarantine Screening

Rearing EAB and its parasitoids. Before scientists could study the biology and host preferences of EAB parasitoids, it was necessary to develop methods to rear both EAB and the parasitoids (see Ch. 8). There were several challenges that needed to be met: (1) parasitoids can potentially be reared all year but EAB is univoltine and has an obligatory diapause as mature larvae, (2) adult EAB eat ash leaves, but leaves are only available in the field in the summer, (3) EAB eggs are needed for rearing the egg parasitoid *O. agrili*, and (4) the larval parasitoids only attack EAB when it is beneath ash bark.

The dilemma of EAB availability was solved by felling ash trees containing large numbers of EAB and storing the logs in cold rooms until the insects were needed. Felling of ash trees could be done (1) during the late summer when logs contained mature larvae appropriate for rearing larval parasitoids or (2) during the winter when overwintering mature fourth-instar larvae were present in their pupation cells (as J-larvae), which quickly developed into EAB adults when warmed.

The need to obtain foliage to feed adults throughout the year was solved by rearing tropical or Shamel ash, *Fraxinus uhdei* (Wenz.) Lingelsh., in greenhouses.

Initially, small ash logs wrapped in curling ribbon were presented to adult beetles for oviposition, but a method of coaxing females to lay eggs on coffee filters was later developed. Eggs on filter papers could then be presented to *O. agrili* for parasitization.

EAB larvae were extracted from the logs by peeling the bark, and these larvae were reinserted in grooves under bark flaps of small ash logs for presentation to *S. agrili* and *T. planipennisi* (Gould et al., 2011). This method was laborious and was later improved upon for mass production (see Ch. 8).

Host specificity testing. Biological control of insect pests using entomophagous natural enemies has generally been considered "natural" and "safe." However recent studies have documented negative impacts on non-target species in some cases (Boettner et al., 2000; Obrycki et al., 2000; Henneman and Marmot, 2001), highlighting the need for pre-release host specificity testing. The specificity of EAB parasitoids imported from China or Russia was estimated in quarantine as part of the process of assembling data needed to apply for release permits. Specificity of agents is summarized below.

(1) Spathius agrili. No-choice host specificity tests with *S. agrili* were conducted in China and the United States to determine possible direct effects on non-target species (Yang et al., 2008). *Spathius agrili* finds hosts to parasitize by hearing sounds or feeling vibrations produced by feeding larvae inside wood. All test larvae, therefore, were presented while feeding inside their natural host trees. In initial no-choice host specificity tests, *S. agrili* did not parasitize wood-boring Lepidoptera, a longhorned beetle (Cerambycidae), or the one *Agrilus* species tested. Of these three species (whose larvae all attack ash), only EAB was parasitized. Further testing was, therefore, confined to members of the genus *Agrilus*, which were hypothesized to potentially be at risk because they were closely related to EAB.

In the United States, we tested the two-lined chestnut borer, *Agrilus bilineatus* Weber, in oak (*Quercus*), and the bronze birch borer, *Agrilus anxius* Gory, in birch (*Betula*), while in China various other local *Agrilus* species were tested.

In no-choice tests, *S. agrili* attacked some species of *Agrilus* other than EAB, but at rates that were significantly lower than for emerald ash borer. In China, *S. agrili* attacked *Agrilus zanthoxylumi* Hou, *Agrilus mali* Matsumura, and *Agrilus inamoenus* Kerremans. No attack occurred on other *Agrilus*

species tested, *Sphenoptera* sp. (Coleoptera: Buprestidae), or *Eucryptorrhynchus chinensis* (Olivier) (Coleoptera: Curculionidae) (Yang et al., 2008).

No-choice tests determine the physiological host range of a parasitoid by giving them no other option but to oviposit on a non-target host. Parasitoid adult orientation to host plants is not part of the test and so this filter is disregarded. To determine the ecological host range of *S. agrili*, olfactometer tests with adults were conducted in China to determine if *S. agrili* were attracted to the plant species harboring the larvae tested in no-choice tests. Naïve, mated *S. agrili* females were placed in vertical y-tube olfactometers and given a choice of leaves and twigs of various host plants or clean air. *Spathius agrili* was only attracted to two ash species (*F. pennsylvanica* and *F. velutina*) and one species of willow (*Salix babylonica* L.) (Yang et al., 2008). Even though some attack occurred on larvae found in *Citrus reticulata* Blanco, *Malus micromalus* Makino, and *Zanthoxylum bungeanum* Maxim in no-choice tests, *S. agrili* females were not attracted to these tree species. In nature, if parasitoids are not attracted to an insect's host tree, they would be unlikely to encounter and parasitize larvae of that non-target species. *Spathius agrili* was attracted to willow leaves, and at least three *Agrilus* species attack willow in the United States: *Agrilus pratensis pratensis* Ratzburg (adults 4 - 6 mm long), *Agrilus politus* Say (adults 5.0 - 8.5 mm long), and *Agrilus quadriguttatus* Gory. These insects are quite small compared with adult EAB, which are 8.5-13.5 mm long. *Spathius agrili* attacks only large EAB larvae, and even mature larvae of *A. pratensis* and *A. politus* are likely too small to be at risk of attack. Thus even if *S. agrili* is attracted to willow in the United States, it is unlikely to encounter any non-target species large enough to be suitable hosts.

Another piece of evidence concerning host specificity was gathered in China by collecting larvae of six *Agrilus* species in the field and then rearing them to determine their parasitoid fauna. A total of 2,074 *Agrilus* larvae of six non-target species were collected and neither *S. agrili* nor *T. planipennisi* were recovered (Yang et al., 2008). Given the combination of evidence from no-choice tests (lower parasitism rates or no attack on non-target *Agrilus* species), olfactometer tests (only attracted to ash and willow), and the lack of

S. agrili reared from other *Agrilus* species in China, it was predicted that release of *S. agrili* would not have adverse direct effects on non-target species in the United States.

(2) Tetrastichus planipennisi. To evaluate the direct effects of *T. planipennisi* on potential non-target North American insect species, no-choice assays were performed in the laboratory with larvae of EAB and eight species of buprestids (five species of *Agrilus* and three of *Chrysobothris*), five cerambycids, two lepidopterans, and one hymenopteran (Liu and Bauer, 2007; Federal Register, 2007). These insects were selected based on (1) the degree of taxonomic closeness to EAB; (2) overlap in habitat and/or niche with EAB; (3) risk to beneficial, threatened, or endangered insects; and (4) feasibility of acquiring or rearing enough larvae to perform replicated assays. *Tetrastichus planipennisi* did not attack any of the seventeen non-target species presented in no-choice tests, it was considered quite host specific, and further testing was not done.

(3) Oobius agrili. To evaluate the direct effects of *O. agrili* on non-target insect species, no-choice assays were performed in the laboratory using eggs of six *Agrilus* species, two cerambycids, and four lepidopterans (Bauer and Liu, 2007; Federal Register, 2007). In no-choice assays, *O. agrili* did not oviposit in eggs of the cerambycids or lepidoptera. *Oobius agrili* may oviposit and develop in *Agrilus* eggs from different species if they have eggs similar in size to those of EAB. Such non-target species include *A. anxius* (bronze birch borer), *A. bilineatus* (two-lined chestnut borer), and *Agrilus ruficollis* (F.) (red-necked cane borer), which are pests of birch, oak, and raspberry, respectively. Paired no-choice and choice assays were then performed for two of the *Agrilus* species that were accepted by *O. agrili* during the no-choice assays. In the choice assays, *O. agrili* preferred eggs of *A. planipennis* (EAB) on ash logs over those of *A. anxius* or *A. ruficollis,* on birch and raspberry, respectively.

(4) Spathius galinae. To evaluate the effects of *S. galinae* on non-target insect species, no-choice and choice host specificity tests were conducted (JRG and JJD unpublished). Fifteen North American species of wood-boring insects were exposed to *S. galinae* to assess the parasitoid's physiological host range. Emphasis was placed on species closely related to the

target pest or those feeding on ash. Thirteen of these fifteen species were wood-boring beetles, one was a clearwing moth (Lepidoptera: Sesiidae) and one a sawfly (Hymenoptera: Cephidae). Of the beetles, five were in the genus *Agrilus,* and were thus closely related to the EAB, and another was in the same family (Buprestidae). Three of the insects tested – the longhorned beetle *Neoclytus acuminatus* (F.), the clear-winged moth (*Podosesia* sp.), and the eastern ash bark beetle, *Hylesinus fraxini* Panz. (Coleop.: Scolytinae) – attack ash as their main host and would be susceptible to parasitism if *S. galinae* accepts any boring insects infesting ash.

Spathius galinae attacked only one species other than the EAB, the gold spotted oak borer (*Agrilus auroguttatus* Schaeffer) in red oak (*Quercus rubra* L.). This species is an invasive borer killing native oaks in California. The rate of parasitism was, however, lower (only 41%) on the non-target host compared to EAB (71%) under test conditions that strongly favored parasitism. *Spathius galinae* did not attack the other three test species that infested red oak, nor did it attack the other *Agrilus* or the three non-*Agrilus* species infesting ash. This level of host specificity is quite high; indeed, higher than that of *S. agrili,* which was approved for release against EAB.

Applying for Release Permits

Applying for permits to release exotic parasitoids against invasive pests in the United States is a complicated process involving review by the North American Plant Protection Organization (NAPPO), whose members include the United States, Canada, and Mexico, by the U.S Fish and Wildlife Service, North American Indian Tribes, the USDA-APHIS permitting unit, and State Departments of Agriculture (Bauer et al., 2014). The application must also be posted in the Federal Register for public comment before APHIS approves or disapproves the application. In January, 2007, USDA scientists applied for environmental release permits for *S. agrili*, *T. planipennisi*, and *O. agrili* in Michigan. The permits were granted at the end of July 2007, and the parasitoid species were released at several sites in Michigan. The application to release *S. galinae* was submitted in March, 2013 and in

November of that year was approved by the NAPPO committee. The final outcome of the permit application to release *S. galinae* in the United States was pending in January 2015.

FIELD ESTABLISHMENT AND EVALUATION OF NATURAL ENEMIES

## Rearing and Release

After release permits were issued in 2007, relatively small numbers of adult *S. agrili*, *T. planipennisi*, and *O. agrili* were released at field sites in Michigan. Releases were expanded to new sites in Ohio and Indiana in 2008, and to Illinois and Maryland in 2009, but only a few hundred adults of each species were released because of the limited rearing capacity of the USDA research laboratories. Despite these limitations, however, establishment of the three species was confirmed within two years at many of these field sites (Bauer et al., 2008, 2011). These early successes resulted in the decision by USDA to initiate an EAB Biological Control Program in 2009 and construct the APHIS EAB Biocontrol Facility in Brighton, Michigan (USDA, 2013). Researchers wrote guidelines to assist land managers with basic information on EAB, the biological control agents, site selection, and methods for release and recovery of the parasitoids (Gould et al., 2013). An online database was also developed where the Guidelines are posted and parasitoids release and recovery data are entered and mapped (mapbiocontrol.org). The EAB biological control agents are now mass-reared for distribution and release throughout the still expanding EAB infestation in North America, and production and release methods continue to be improved (see Ch. 8).

In 2009, the APHIS EAB Biocontrol Facility concentrated on rearing *S. agrili*, and 10,000 adult females of that species were reared and released (Fig. 1) (J. Lelito, personal communication). In 2010, a concerted effort was made to increase production of *T. planipennisi* (Fig. 2), and production of the egg parasitoid, *O. agrili,* was greatly increased in 2011 (Fig. 3). Production of these parasitoids increased
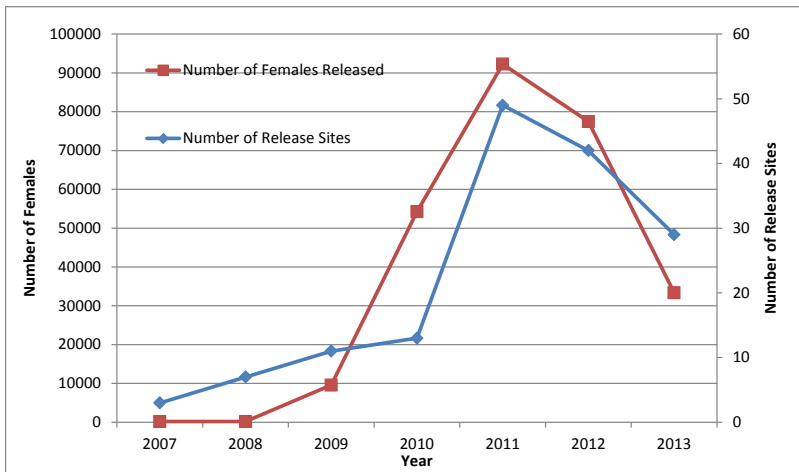
Figure 1. Number of female *Spathius agrili* released against emerald ash borer 2007-2013 and the number of release sites in the United States.
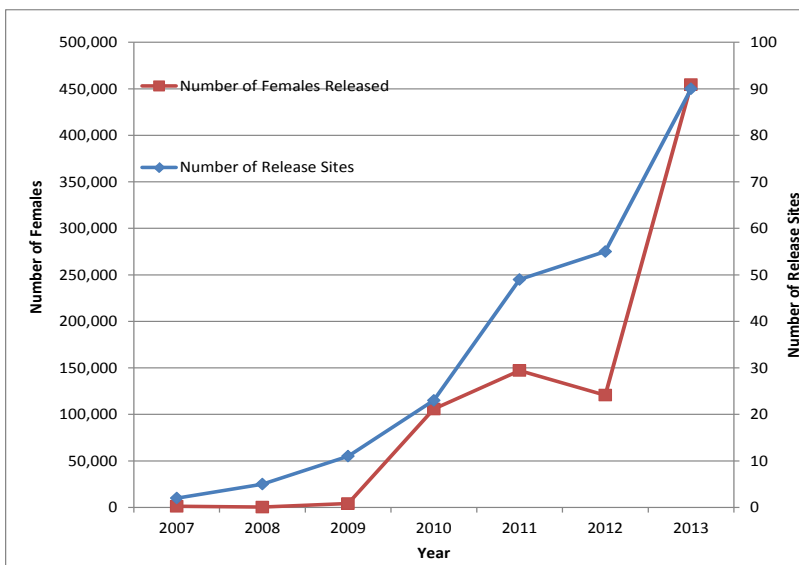
Figure 2. Number of female *Tetrastichus planipennisi* released against emerald ash borer 2007-2013 and the number of release sites in the United States.
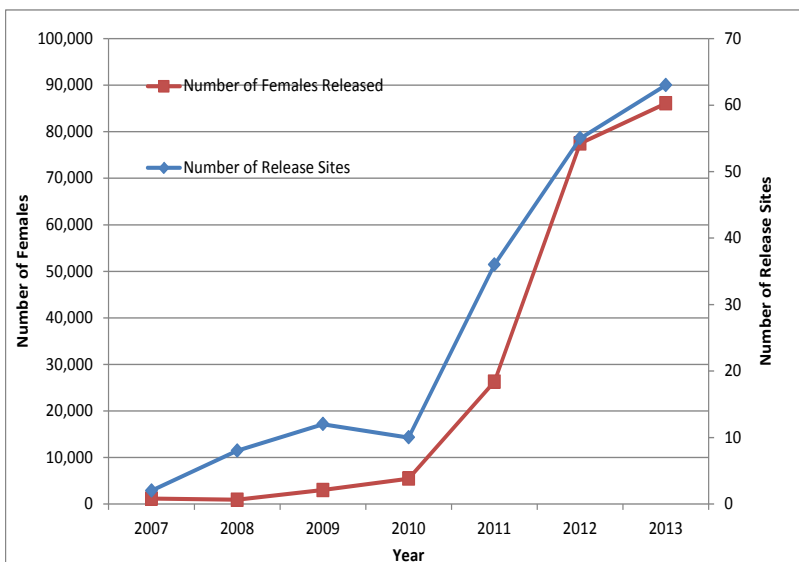
Figure 3. Number of female *Oobius agrili* released against emerald ash borer 2007- 2013 and the number of release sites in the United States.

in subsequent years, with the exception of *S. agrili*, which was not released in northern states after 2012 (see the Establishment section for explanation). The increased production was necessary because each year at least two more states initiated releases of EAB parasitoids (Table 1), so that by 2013 seventeen states were conducting releases. In 2014 Colorado, Georgia, New Hampshire, and New Jersey discovered populations of EAB and releases in those states were set to begin that year.

To improve parasitoid production and release efficiency, the APHIS EAB Biocontrol Facility began modifying the shipment and release methods for the three EAB biological control agents. Before 2012, parasitoids were released as adults. This was done by rearing them to the adult stage in the laboratory, consolidating them into plastic cups provisioned with honey, and shipping them inside coolers to cooperators who released them onto the trunks of EAB-infested ash trees. In 2012, APHIS began shipping parasitoids as mature larvae or pupae for self-emergence in the field from cups containing parasitized EAB eggs for *O. agrili* or ash bolts containing EAB larvae parasitized with either *T. planipennisi* or *S. agrili*. This change began for *O. agrili* in 2012 and for the two larval parasitoid species in 2013. Further research is needed to evaluate the success of the newer release methods.

Establishment

Sampling methods. Sampling EAB to determine whether or not larvae or eggs are parasitized poses quite a challenge. EAB eggs are small and laid between layers of bark and in bark crevices. EAB larvae feed beneath the bark of ash trees, and to recover them the bark must be peeled off to expose the larvae. If the goal is to determine the percentage of EAB that are parasitized, one must search ash trees for EAB eggs and larvae.

However, if the goal is only to confirm establishment, several additional methods have been developed (Bauer et al., 2012; Duan et al., 2011, 2012b). For detection of *O. agrili*, laboratory-reared EAB eggs can be placed in the field under bark flaps, on small ash logs, or in cups on paper. For the larval parasitoids, EAB larvae can be inserted in small ash

Table 1. States initiating releases of EAB parasitoids by year.

Year	States Inititiating Releases
2007	Michigan
2008	Ohio, Indiana
2009	Illinois, Maryland
2010	West Virginia, Kentucky, New York, Wisconsin
2011	Pennsylvania, Minnesota, Virginia
2012	Missouri. Tennessee
2013	Massachusetts, North Carolina, Connecticut

logs and placed in the field. Creating these "sentinel" eggs or larvae, however, requires either rearing adult EAB or collecting larvae for insertion in the sentinel logs. EAB parasitoids have been recovered at several sites using sentinel logs, and egg sentinel logs were used to document the phenology of *O. agrili* activity in the field in Michigan (Abell et al., 2011).

Both larval and egg parasitoids can also be recovered by collecting logs or bark samples and placing them in cardboard rearing tubes fitted with collection jars. The emerging parasitoid adults are attracted to the light in the jar and essentially collect themselves.

Finally, adult parasitoids can be recovered in the field using yellow pan traps filled with a solution of propylene glycol (Bauer et al., 2013). These traps are inexpensive to produce and easy to deploy, but distinguishing the biological control agents from similar native species requires individuals trained in insect taxonomy and identification.

Pheromones have been identified for both *S. agrili* and *T. planipennisi* (Bauer et al., 2011; Cossé et al., 2012), and the use of pheromone lures is being investigated as a method to increase the efficacy of yellow pan traps.

Reproduction, overwintering, and establishment. For the introduced parasitoids to successfully

control EAB, they must find conditions suitable for reproduction in the field, survive cold winter temperatures, and persist from year to year. The three introduced parasitoids released as EAB biological control agents have several generations per year, and their presence in the field must coincide with the availability of the stages suitable for parasitism (eggs for *O. agrili* and larvae for *S. agrili* and *T. planipennisi*). Throughout their adult lives, the parasitoid must also find sources of nourishment such as nectar or honeydew.

Spathius agrili was found parasitizing 18% of the EAB larvae sampled the spring following the release of 175 females in 2007 in southern MI (JRG unpublished). However, samples collected from 40 ash trees the following year revealed not a single parasitoid. At another site, parasitism one year after release was 45%. Two broods were discovered each of the following two years, but parasitism by *S. agrili* remained consistently low. At six more intensively sampled study sites in Michigan, *S. agrili* was recovered in yellow pan traps, but only two EAB larvae parasitized by this species were recovered after 2-5 years of sampling (Duan et al., 2013b). *Spathius agrili* also does not seem to have persisted in Maryland or Ohio. One possible explanation for this apparent lack of persistence is that the population of *S. agrili* reared for release in the United States originated from Tianjin, China. The latitude of this city is near the 39th parallel, and the climate there is a better match for the central (north-south) rather than the northern United States. *Spathius agrili* can successfully overwinter in the midwestern United States, so cold is probably not the limiting factor. Perhaps there is a problem with synchrony between the emergence of adult *S. agrili* and availability of the mature EAB larvae that they need to attack. Based on this observation, the EAB Biocontrol Program decided in 2013 to cease releasing *S. agrili* above the 40th parallel in North America. If *S. galinae* is approved for release, we anticipate that it will be better synchronized with its EAB host in the more northern states. *Spathius agrili* has also been recovered in Illinois, Indiana, Pennsylvania, New York and Tennessee, and we are especially interested in whether *S. agrili* will persist in Tennessee, where the climate is more similar to the parasitoid's native range in China.

Oobius agrili has been recovered in Michigan, Ohio, Indiana, Pennsylvania, Maryland, and New York. At two sites in Michigan, parasitism by *O. agrili* increased from 5% one year following release to 20% two years later (Duan et al., 2011, 2012b; Abell et al., 2011). This species was also recovered at non-release locations at least 800 m from the release site two years after release. Parasitized eggs were found on 73% of trees in the release plots and 25% of the trees in the control plots (Abell et al., 2014), providing evidence that *O. agrili* populations are slowly building and dispersing in Michigan.

Establishment and spread of *T. planipennisi* is even more impressive. At six intensively studied sites in Michigan, 92% of the trees at the release sites contained at least one brood of *T. planipennisi* four years after release, and parasitism levels increased steadily to an average of over 20% (Duan et al., 2013b). Parasitism by *T. planipennisi* at the six control sites (at least 1 km away) also increased yearly to an average level of 13% after four years. The rearing facility in Brighton, Michigan, often finds *T. planipennisi* in trees harvested far from known release locations. It is not known whether *T. planipennisi* is dispersing so well on its own or by human movement of infested firewood, or both. *Tetrastichus planipennisi* has also been recovered from Illinois, Indiana, Ohio, New York, Maryland, Wisconsin, and Minnesota.

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CHAPTER 6: BIOLOGY OF EMERALD ASH BORER PARASITOIDS

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INTRODUCTION

The emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an invasive beetle introduced from China (Bray et al., 2011), was identified as the cause of ash (*Fraxinus* spp.) mortality in southeast Michigan and nearby Ontario in 2002 (Haack et al., 2002; Federal Register, 2003; Cappaert et al., 2005). Although eradication was attempted for several years after the beetle's discovery, it continued to spread throughout North America, killing ash trees in urban, forested, and riparian areas. In an effort to conserve native species of *Fraxinus*, researchers continue to evaluate integrated pest management methods that include the use of classical biological control, systemic insecticides, and the development of resistant cultivars (Herms and McCullough, 2014).

Surveys of EAB populations in recently invaded areas of North America revealed a low prevalence of native generalist parasitoids, mainly species in *Atanycolus* and *Spathius* (Hymenop.: Braconidae), and *Phasgonophora sulcata* Westwood (Hymenop.: Chalcididae) (Bauer et al., 2004; Lindell et al., 2008; Duan et al., 2009, 2012a, 2013a). In regions of China where EAB is native, specialist EAB parasitoids were recovered (Liu, H-Q. et al., 1996; Liu, H-P. et al., 2003, 2007; Zhang et al., 2005; Yang et al., 2005). The most promising of these parasitoids for EAB biocontrol in North America were *Oobius agrili* Zhang and Huang (Hymenop.: Encyrtidae), *Tetrastichus planipennisi* Yang (Eulophidae), and *Spathius agrili* Yang (Hymenop.: Braconidae).

Following research on the biology, host specificity, and impacts of these parasitoid species on EAB population dynamics in China, researchers proposed their release as EAB biocontrol agents in the continental United States in an Environmental Assessment (Federal Register, 2007). After a public comment period in 2007, regulatory agencies involved in biological control risk-benefit analyses approved trial releases of the three parasitoid species in Michigan, permits were issued, and releases began (Bauer et al., 2008, 2009, 2014, in press). Establishment of the introduced parasitoids was confirmed within a year of their first release, leading to the decision by USDA to initiate the EAB Biocontrol Program, construction of an EAB-parasitoid rearing facility in Brighton, Michigan, and development of an online database where parasitoids can be requested and data on parasitoid releases, recoveries, and mapping are stored (USDA FS, 2009; Bauer et al., 2010ab; MapBioControl, 2014). As a result, EAB biological control agents are being released in other states with known EAB infestations. In addition, another larval parasitoid of EAB from the Russian Far East, *Spathius galinae* Belokobylskij (Hymenop.: Braconidae), is being considered for release in the future (Belokobylskij et al., 2012; Duan et al., 2012b).

To improve the integrated pest management of EAB in forest ecosystems using classical biological control, researchers have been studying the introduced and native natural enemies of EAB populations at long-term study sites in Michigan and other states (Bauer et al., in press; Duan et al., 2010, 2012a, 2013b, 2014a; Jennings et al., 2014). In this chapter, we will review the literature on the biology of key parasitoids known to attack EAB in North America and Asia.

BIOLOGY OF NATIVE OR SELF-INTRODUCED PARASITOIDS ATTACKING EAB IN NORTH AMERICA

Several native parasitoids and one self-introduced exotic species are known to attack EAB larvae at field sites in North America; no native EAB egg parasitoids are known (Table 1). In general, the EAB larval parasitoids reported in North America are parasitoids of *Agrilus* spp., although several species also parasitize the larvae of other groups of wood-boring insects (Gibson, 2005; Duan et al., 2009; Taylor et al., 2012). Rates of larval parasitism by these species are generally low (<5%) during the initial phase of the EAB invasion; however, there are reports in Michigan of increasing larval parasitism by parasitoids of other coleopteran woodborers (Cappaert and McCullough, 2009; Duan et al., 2012a, 2014a).

The most prevalent native parasitoids of EAB are several braconids in the genus *Atanycolus* and

Table 1. List of reported hymenopteran parasitoids attacking emerald ash borer larvae or eggs.

Name	Family	Parasitoid biology	Reported Range
Sclerodermus pupariae	Bethylidae	gregarious larval ectoparasitoid	China
Atanycolus cappaerti	Braconidae	solitary larval ectoparasitoid	Michigan
Atanycolus disputabilis	"	"	Northeastern North America
Atanycolus hicoriae	"	"	Northeastern North America
Atanycolus nigropopyga	"	"	Northeastern North America
Atanycolus nigriventris	"	"	Russian Far East
Atanycolus simplex	"	"	Northeastern North America
Spathius agrili[1]	"	gregarious larval ectoparasitoid	China, Northeastern US
Spathius floridanus[2] (= *Spathius simillimus*)[2]	"	"	Northeastern North America
Spathius galinae[3]	"	"	Russian Far East, South Korea
Spathius laflammei (= *Spathius benefactor*)	"	"	United States
Spathius polonicus	"	"	Europe, Moscow
Leluthia astigmata	"	solitary larval endoparasitoid	United States
Phasgonophora sulcata	Chalcididae	solitary larval endoparasitoid	Northeastern North America
Oobius agrili[1]	Encyrtidae	solitary parthenogenic egg parasitoid	China, United States
Oobius sp.	"	"	Russian Far East
Oencyrtus sp.	"	solitary egg parasitoid	China
Tetrastichus planipennisi[1,4]	Eulophidae	gregarious larval endoparasitoid	China, Russian Far East, North America
Tetrastichus sp.	"	"	South Korea
Balcha indica	Eupelmidae	solitary parthenogenic, larval ectoparasitoid	Southeast Asia, Northeastern United States
Eupelmus sp.	"	solitary ectoparasitoid	Northeastern North America
Cubocephalus sp.	Ichneumonidae	solitary larval ectoparasitoid	Northeastern North America
Dolichomitus sp.	"	"	Northeastern North America
Orthizema sp.	"	"	Northeastern North America

[1] introduced as EAB biological control agents in the United States starting in 2007
[2] recent evidence suggests these are separate species (JPL, J. Strazanac, N. Havill, unpublished data)
[3] in 2015, proposed for release as an EAB biological control agent in the United States
[4] introduced as EAB biological control agent in Canada starting in 2013

the chalcidid *Phasgonophora sulcata* Westwood (Bauer et al., 2008; Duan et al., 2009, 2013a, 2014a). In the genus *Atanycolus, A. cappaerti* Marsh and Strazanac and *A. hicoriae* Shenefelt are the most common species found attacking EAB in Michigan, Ohio, or Pennsylvania, but *A. simplex* Cresson, *A. nigropopyga* Shenefelt, and *A. disputabilis* (Cresson) are also reported (Bauer et al., 2008; Cappaert and McCullough, 2009; Duan et al., 2013a). Other less common parasitoids include (1) several braconid species – *Spathius floridanus* Ashmead, *S. simillimus* Ashmead (see taxonomic changes below in section on *Spathius* biology), *S. laflammei* (= *Spathius benefactor* Matthews), and *Leluthia astigmata* (Ashmead); (2) several unknown ichneumonids in *Dolichomitus, Orthizema,* and *Cubocephalus*; and (3) two eupelmids – *Eupelmus* sp. and *Balcha indica* (Mani & Kaul)

(Bauer et al., 2005, 2008; Duan et al., 2009, 2013a, 2014a; Kula et al., 2010). These parasitoids are native except for *B. indica*, which is from Southeast Asia and is self-naturalized in the eastern United States where it attacks a range of wood-boring beetles (Gibson, 2005).

Atanycolus spp. (Braconidae)

Marsh et al. (2009) reports 11 native species of *Atanycolus* in North America, which parasitize the larvae of *Agrilus* species or those of other wood-boring beetles. The five *Atanycolus* species reported from species of *Agrilus* (Taylor et al., 2012) are solitary, ectoparasitic idiobionts of late-stage larvae that complete one or two generations in northern regions of the United States (Fig. 1a-d). Many of the *Atanycolus* adults reared in the laboratory from

Figure 1. *Atanycolus* species life stages. (a) *Atanycolus* adult ovipositing onto an EAB larva in the trunk of an ash tree. (Photo credit: Houping Liu); (b) *Atanycolus* egg on an EAB larva photographed though a dissecting microscope (45X). (Photo credit: Deborah Miller); (c) *Atanycolus* larva feeding on an EAB larva in its gallery. (Photo credit: Deborah Miller); (d) *Atanycolus* cocoon containing a pupa with the remnant of its EAB larval host (to the left of the cocoon) in an EAB gallery. (Photo credit: David Cappaert)

EAB larvae are relatively large wasps (5-7 mm long) with long ovipositors (4-6 mm) (Marsh et al., 2009). Consequently, they can parasitize EAB larvae in mature ash trees (>57 cm diameter at breast height [DBH]) with thick outer bark (up to 9 mm thick) (Abell et al., 2012). The biology of *A. cappaerti*, a recently described species found parasitizing EAB in Michigan, is best known and is typical of other *Atanycolus* species (Cappaert and McCullough, 2009; Marsh et al., 2009).

The biology of *A. cappaerti* is reported from a field study in southern Michigan in 2007 and 2008 (Cappaert and McCullough, 2009; Tluczek et al. 2010). The life cycle of *A. cappaerti* is generally well synchronized with EAB in Michigan, with increasing numbers of cocoons found throughout the summer and fall from newly developing EAB larval hosts. By the end of October, *A. cappaerti* larvae are found parasitizing medium to large EAB larvae that are still actively feeding in the phloem. *Atanycolus cappaerti* also parasitizes the larvae of *Agrilus liragus* Barter & Brown and *Agrilus bilineatus* (Weber), demonstrating at least a genus-level host range for this parasitoid in Michigan forests (Cappaert and McCullough 2009). Due to similarities in the morphology and biology of *A. cappaerti* and *A. hicoriae,* another parasitoid of EAB larvae in Michigan, these two species are combined as "*Atanycolus* spp." for studies on EAB population dynamics (e.g., Duan et al., 2013a, 2014a).

Species of *Atanycolus* (Fig. 1a-d) overwinter as mature larvae or prepupae inside cocoons spun in EAB galleries during the fall. Adult emergence begins in early June, and these adults parasitize overwintered EAB larvae. Most first generation *Atanycolus* larvae complete their development in about one month, with emergence occurring in early to mid-July. These wasps parasitize the current year's EAB larvae. The longevity of female wasps held in the laboratory averaged 32 days (JJD, unpublished data). As reported for other braconid parasitoids of *Agrilus*, some first generation larvae may enter diapause, overwinter, and emerge as adults the following spring.

Spathius spp. (Braconidae)

Species of the genus *Spathius* are gregarious ectoparasitic idiobionts of various coleopteran families including Cerambycidae, Buprestidae, Scolytinae, Curculionidae, Bostrichidae, and Anobiidae (Marsh and Strazanac, 2009). In North America, several species of *Spathius* are found attacking late-instar EAB larvae (Bauer et al. 2004; Duan et al., 2009) (Table 1).

Spathius floridanus (Fig. 2a,b) and *S. simillimus* are the most common *Spathius* species found attacking EAB in Michigan (Bauer et al., 2004), while *S. laflammei* is the common species in western Pennsylvania (Duan et al., 2009) (Table 1). Following the initial identifications of the two species attacking EAB in Michigan, Marsh and Strazanac (2009) merged these into *S. floridanus*. Current evidence, however, suggests that *S. floridanus* and *S. simillimus* are distinct species (JPL, J. Strazanac, N. Havill, unpublished data). Although published literature is lacking on the biology of these native *Spathius*, laboratory studies in Michigan found *Spathius* adults emerged in late spring and early summer, and completed one or two generations before fall temperatures induced diapause in the remaining larvae; when reared in the laboratory at 25-27 °C, *Spathius* species completed a generation in 28-32 days (JPL, unpublished data).

Phasgonophora sulcata (Chalcididae)

Phasgonophora sulcata (Fig. 3a-d), native to eastern North America, is a solitary endoparasitic koinobiont of *Agrilus* larvae and completes one generation per year. It has been reared from *A. anxius, A. bilineatus, A. liragus,* and more recently from EAB in the United States and Canada (for review see Taylor et al., 2012). The emergence of *P. sulcata* adults lags about two weeks behind that of EAB adults (Roscoe, 2014). In the field in southern Michigan, these relatively large wasps (~8 mm long) are readily observed during late June through July seeking host larvae in EAB-infested ash trees. By sequential larval dissections throughout the season, we have observed the hatch and slow development of *P. sulcata* eggs and larvae in the posterior region of the host hemocoel. Preliminary studies suggest *P. sulcata* parasitizes first or second instar EAB larvae; pupation occurs the following spring inside host prepupae (LSB, unpublished data).
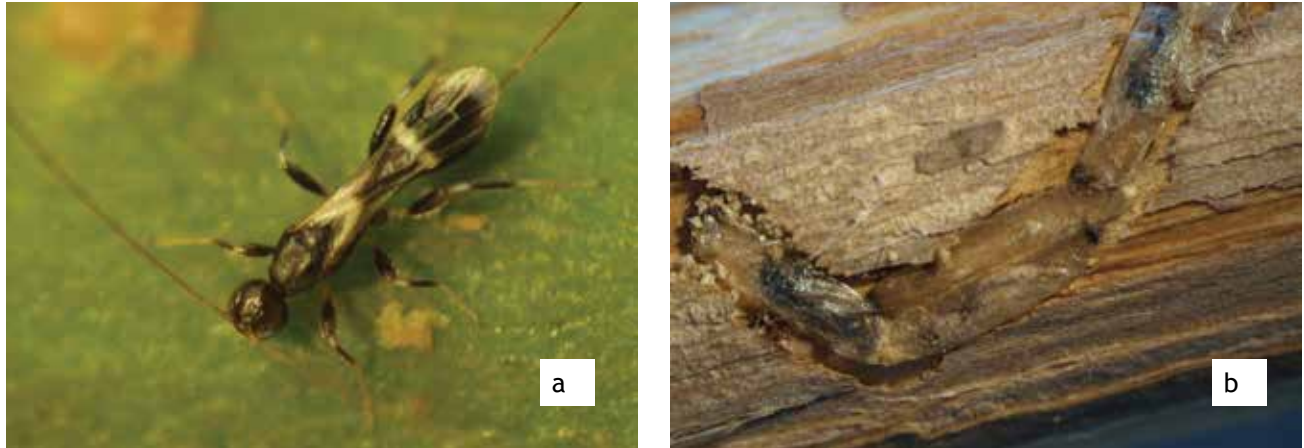
Figure 2. *Spathius floridanus* life stages: (a) *S. floridanus* adult searching for EAB larvae in a small ash log in the laboratory. (Photo credit: Jian Duan); (b) *S. floridanus* cocoons with pupae in an EAB larval gallery. (Photo credit: Jian Duan)
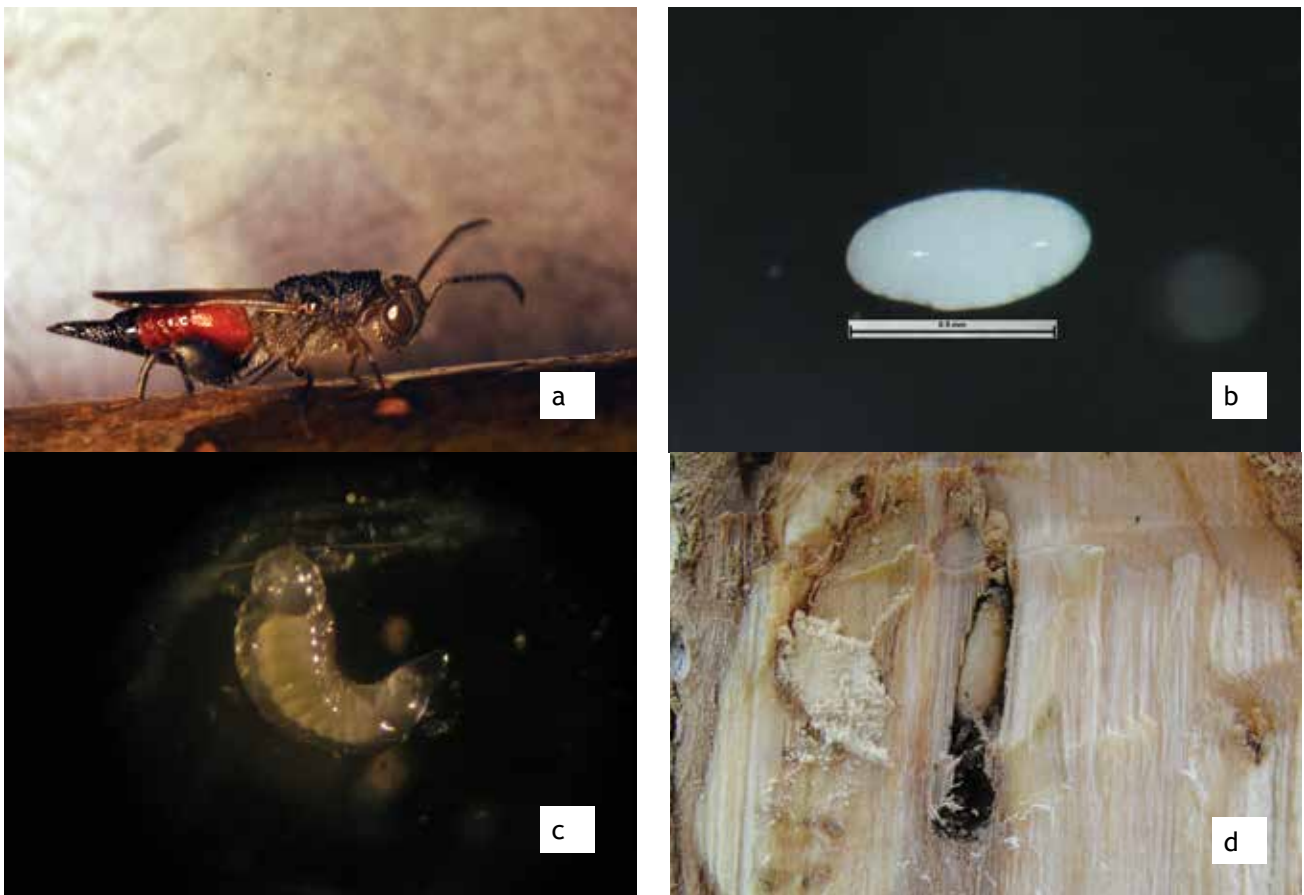
Figure 3. *Phasgonophora sulcata* life stages: (a) *P. sulcata* adult searching for EAB larva in a small ash log in the laboratory. (Photo credit: Deborah Miller); (b) *P. sulcata* egg dissected from a field-collected EAB larva. (Photo credit: Deborah Miller); (c) *P. sulcata* larva dissected from the posterior hemocoel of a field-collected EAB larva. (Photo credit: Deborah Miller); (d) *P. sulcata* cocoon in an EAB pupal chamber in the trunk of an ash tree. (Photo credit: Leah Bauer)

Balcha indica (Eupelmidae)

Balcha indica (Fig. 4a-c), native to Southeast Asia and naturalized in eastern United States, is occasionally found parasitizing EAB and other wood-boring beetles (Gibson, 2005). It is a solitary, ectoparasitic and parthenogenic idiobiont that parasitizes EAB larvae, prepupae, and pupae (Bauer et al., 2004; Duan et al., 2009). Adult females reared from woodborers in the United States vary in size from 3 to 8 mm long (Gibson, 2005). Duan et al. (2011a) studied its biology in the laboratory at 25 ºC using adult females reared from parasitized EAB larvae collected in Pennsylvania (Duan et al., 2009). They found the generation time of *B. indica* averaged 83 days (range 47-129), which is slow compared to the development time of other EAB ectoparasitoids reared under similar conditions. Female fecundity averaged 36 eggs during an average 59 day life span. These laboratory findings support field observations of one unsynchronized generation per year attacking immature EAB infested ash trees in Michigan, Pennsylvania, and Maryland (Duan et al., 2011a). As an established parasitoid of EAB and other woodborers in the United States, *B. indica* will continue playing a role in suppressing EAB population densities in North America (Duan et al., 2014a).

BIOLOGY OF PARASITOIDS ATTACKING EAB IN EURASIA

Several hymenopteran parasitoid species attack EAB larvae in Asia, as does one recently discovered attacking EAB in Europe (Table 1). In Asia, rates of EAB larval parasitism are consistently higher than those reported for EAB in North America. *Tetrastichus planipennisi* (Hymenop.: Eulophidae) is the dominant parasitoid of EAB larvae in northeast China (Liu, H-P. et al., 2003, 2007; Yang et al., 2006). This parasitoid was also found attacking EAB in the Khabarovsk and Vladivostok regions of the Russian Far East (Duan et al. 2012b). In South Korea, an unidentified species of *Tetrastichus* was found parasitizing EAB larvae (Williams et al., 2010). Other larval parasitoids of EAB in Asia are in the family Braconidae. *Spathius agrili* is the most prevalent parasitoid of EAB larvae in the

Figure 4. *Balcha indica* life stages: (a) *B. indica* adult reared from an EAB prepupae in laboratory. (Photo credit: Houping Liu); (b) *B. indica* larva parasitizing an EAB larva in gallery. (Photo credit: Houping Liu); (c) *B. indica* cocoon with remnant of EAB host larva in gallery. (Photo credit: Houping Liu)

vicinity of Tianjin, China, southeast of Beijing. It is also found sporadically in the northeastern provinces (Xu, 2003; Liu, H-P. et al., 2003; Yang et al., 2005; Wang et al., 2010). In the vicinity of Vladivostok in the Russian Far East, both *Spathius galinae* Belokobylskij and *Atanycolus nigriventris* Vojnovskaja-Krieger parasitize EAB larvae (Williams et al., 2010; Belokobylskij et al., 2012; Duan et al., 2012b). *Spathius galinae* was also reported attacking EAB in South Korea. More recently, *Spathius polonicus* Niezabitowski, a braconid native to Europe, was discovered attacking EAB larvae in Moscow (Orlova-bienkowskaja and Belokobylskij, 2014). Another hymenopteran parasitoid, *Sclerodermus pupariae* Yang and Yao (Bethylidae) attacks EAB larvae and pupae in the region of Tianjin, China (Wu et al., 2008; Wang et al., 2010; Tang et al., 2012; Yang et al., 2012). Due to a broad host range and tendency to sting humans, this species was not considered for EAB biological control in North America.

Few egg parasitoids are known to attack EAB in Asia (Table 1). *Oobius agrili* (Hymenop.: Encyrtidae) is the most widespread parasitoid of EAB eggs and was first discovered in 2004 in Jilin province, China (Zhang et al., 2005). It is now known from other provinces in northeast China (LSB & JJD, unpublished data). More recently, a closely related species of *Oobius* was discovered in the Russian Far East (JJD, unpublished data) and an undescribed species of *Oenycyrtus* (Hymenop.: Encyrtidae) was reared from EAB eggs collected in Jilin province, China (LSB, unpublished data).

Oobius agrili (Encyrtidae)

Discovered in northeast China, *O. agrili* is a solitary parthenogenic parasitoid of EAB eggs. Due to its importance as a natural enemy of EAB in northeast China, where egg parasitism averaged 44% (Liu, H-P. et al., 2007), *O. agrili* was approved for use as a biological control agent of EAB in the United States in 2007 (Federal Register, 2003). Establishment and spread is confirmed in Michigan and other states (Duan et al., 2011b, 2012c; Bauer et al., 2013, 2014, in press). At six EAB biological control study sites in Michigan where researchers began monitoring EAB mortality following the first parasitoid releases in 2007, the level of egg parasitism by *O. agrili* increased from 0.7% to 22%

from 2008 to 2012 (Abell et al., 2014).

Oobius agrili (Fig. 5a-d) overwinter as diapausing prepupae inside EAB eggs, and adult eclosion is well synchronized with the oviposition period of EAB, starting in late June and continuing into September in China and Michigan (Liu, H-P. et al., 2007; Bauer and Liu, 2007; Abell et al., 2011). When *O. agrili* completes two generations per year, ~80% of the first generation progeny emerge and parasitize newly laid EAB eggs, whereas ~80% of the second generation enter obligate diapause for the winter. Moreover, the number of progeny entering diapause also increases as the female ages, and diapause may also be induced by exposure of adults to short day length (LSB, unpublished data). Consequently, *O. agrili* completes one or two generations per year (Liu, H-P. et al., 2007). Because this species is parthenogenic, only females are reared and released in the United States for EAB biological control. Males were recovered from parasitized EAB eggs collected in 2005 Jilin province, China; the sex ratio of adults reared from that sample was 15:1 (female: male). In the laboratory when reared at 24ºC, non-diapausing *O. agrili* complete one generation every 28 to 34 days, with an average fecundity of 80 progeny per wasp. The average longevity of females exposed to eggs in the laboratory is 34 days (LSB, unpublished data).

Tetrastichus planipennisi (Eulophidae)

Native to regions of China and the Russian Far East, *T. planipennisi* is a gregarious endoparasitic koinobiont of EAB larvae. Due to its importance as a natural enemy of EAB in regions of Asia where larval parasitism averaged 22% (Liu, H-P. et al., 2007), *T. planipennisi* was approved for biological control of EAB in the United States in 2007 (Federal Register, 2007) and Canada in 2013 (CFIA, 2013). Its establishment and spread was confirmed in Michigan and other states (Bauer et al., 2014, in press; Gould et al. 2011a, 2013; Duan et al. 2013b, 2014a). At the six Michigan EAB-biological control study sites where releases began in 2007, researchers found EAB larval parasitism by *T. planipennisi* increased from 1.2% to 21% from 2008 to 2012 (Duan et al., 2013b).

Figure 5. *Oobius agrili* life stages. (a) *O. agrili* adult ovipositing in an EAB egg, as observed through a dissecting microscope. (Photo credit: Deborah Miller); (b) *O. agrili*-parasitized EAB egg (left) with early symptoms of *O. agrili* development, characterized by darkening coloration of egg, breathing tube and air bubble; a healthy, unparasitized EAB egg (right). (Photo credit: Deborah Miller); (c) *O. agrili* prepupa (left) and young, developing pupa (right) dissected from a field-collected parasitized EAB egg. (Photo credit: Houping Liu); (d) *O. agrili*-parasitized EAB eggs range in color from black (left) to tan (right, for comparison). Adult *O. agrili* chew a round exit hole on the dorsal surface of the egg to emerge. *O. agrili* meconium pellets are excreted prior to pupation and remain visible as dark beads inside a lighter colored parasitized egg (right). (Photo credit: Deborah Miller)

Tetrastichus planipennisi (Fig. 6a-f) lacks obligate diapause and overwinters as prepupae inside host galleries or as young larvae inside host larvae. In northeast China and Michigan, adult emergence begins in April or May when females begin parasitizing overwintered EAB larvae ranging in age from second through fourth instar (Liu, H-P. et al., 2007; Duan et al., 2013a). After maturation, pupation, and eclosion, which all occur in the host gallery the following spring, adults chew small, round exit holes in the tree bark, emerge, and disperse. From field collections in Jilin province, China, *T. planipennisi* completed about four generations per year (Liu, H-P. et al., 2007). An average of 35 individual wasps (range 5 to 122) developed within a single host larva, with a sex ratio of 2.5:1 (female: male) (Liu and Bauer, 2007; Liu, H-P. et al., 2007).

Tetrastichus planipennisi is a relatively small parasitoid (3 to 4 mm long) and may be more effective at parasitizing EAB larvae in small ash trees (<12 cm DBH) with thin bark, due to its short ovipositor

(2.0 to 2.5 mm long), than in large ash trees (Yang et al., 2006; Abell et al., 2012). The rate of spread of *T. planipennisi* in Michigan was estimated at >5 km per year between 2007 and 2010 (LB and JL, unpublished data). In the laboratory, *T. planipennisi* completes one generation every 27 days at 25 °C, has a sex ratio of 4:1 (female: male), has an average realized fecundity of ~45 female progeny per female; and has an average female longevity of 42 days (Ulyshen et al., 2010; Duan et al., 2011b; Duan and Oppel, 2012).

Spathius agrili (Braconidae)

Known mainly from China southeast of Beijing, *S. agrili* is a gregarious ectoparasitic idiobiont of late-instar EAB larvae (Xu, 2003; Liu, H-P. et al., 2003; Yang et al., 2005, 2010). *Spathius agrili* was approved for biological control of EAB in the United States in 2007 (Federal Register, 2007); however, in 2013 APHIS restricted its release to regions below the 40th parallel because of a failure to establish further north
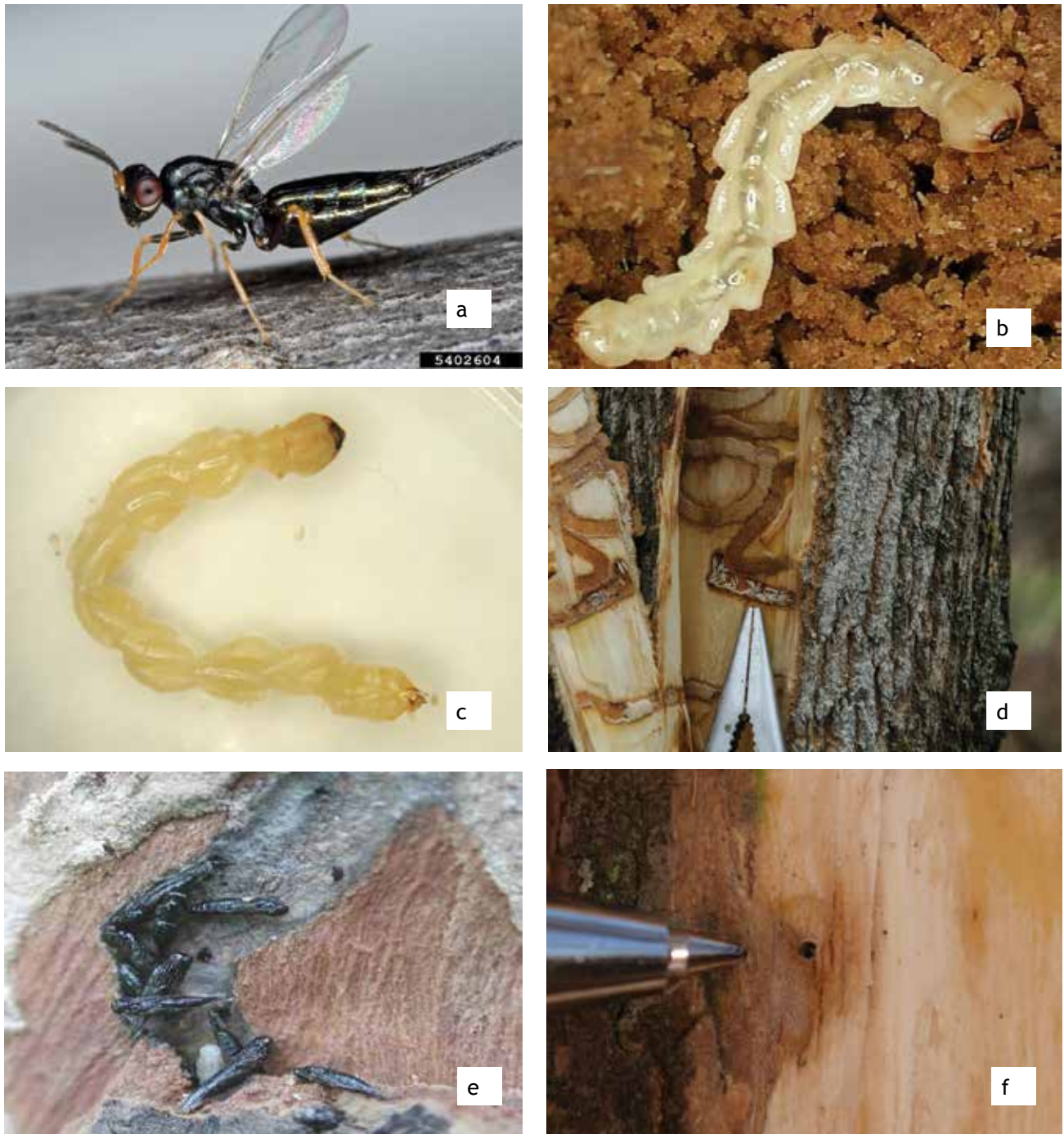
Figure 6. *Tetrastichus planipennisi* life stages (a) adult female (Photo credit: David Cappaert; (b) Young *T. planipennisi* larvae are visible inside an emerald ash borer larva. (Photo credit: Deborah Miller); (c) Mature *T. planipennisi* larvae completing development inside an emerald ash borer larva. (Photo credit: Houping Liu); (d) Fully mature *T. planipennisi* larvae break free of emerald ash borer larval skin and pupate in the larval gallery under the tree bark. (Photo credit: Clifford Sadof). (e) *T. planipennisi* pupae in emerald ash borer larval gallery. (Photo credit: Houping Liu). (f) After eclosion to the adult stage in emerald ash borer galleries, adult *T. planipennisi* chew an exit hole to emerge from the ash trees (with tip of pen for scale). (Photo credit: Leah Bauer)

(Bauer et al., 2014, in press; Gould et al., 2011a,b; 2013;USDA APHIS/FS/ARS, 2013).

Spathius agrili (Fig. 7a-d) overwinter as mature larvae or prepupae in silken cocoons, emerge as adults in July and August and complete one or two generations per year, with a clutch size of 5 to 6 individuals per EAB larva (Wang et al., 2006, 2008; Gould et al., 2011a). In the laboratory when reared at 25:20 ºC (day:night temperature cycles) and 16:8 (light:dark photoperiod), the sex ratio of *S. agrili* averaged 4:1 (female: male), and an average fecundity of ~40 female progeny per female. The average longevity of females is 61 days (Gould et al., 2011a).

Spathius galinae (Braconidae)

Spathius galinae (Fig. 8), recently discovered in the Russian Far East and reported in South Korea, is a gregarious, ectoparasitic idiobiont of EAB larvae (Williams et al., 2010; Belokobylskij et al., 2012; Duan et al., 2012b). When reared in the laboratory at 25 ℃ and 16:8 (light:dark photoperiod), *S. galinae* develops from egg to adult in about a month and completes with one generation per year; female longevity averages 49 days and produce an average of 31 progeny in clutches ranges in size from 5 to 12 individuals per EAB larva (Duan et al. 2014b). Due to better climate matching of the Russian Far East with northern regions of the
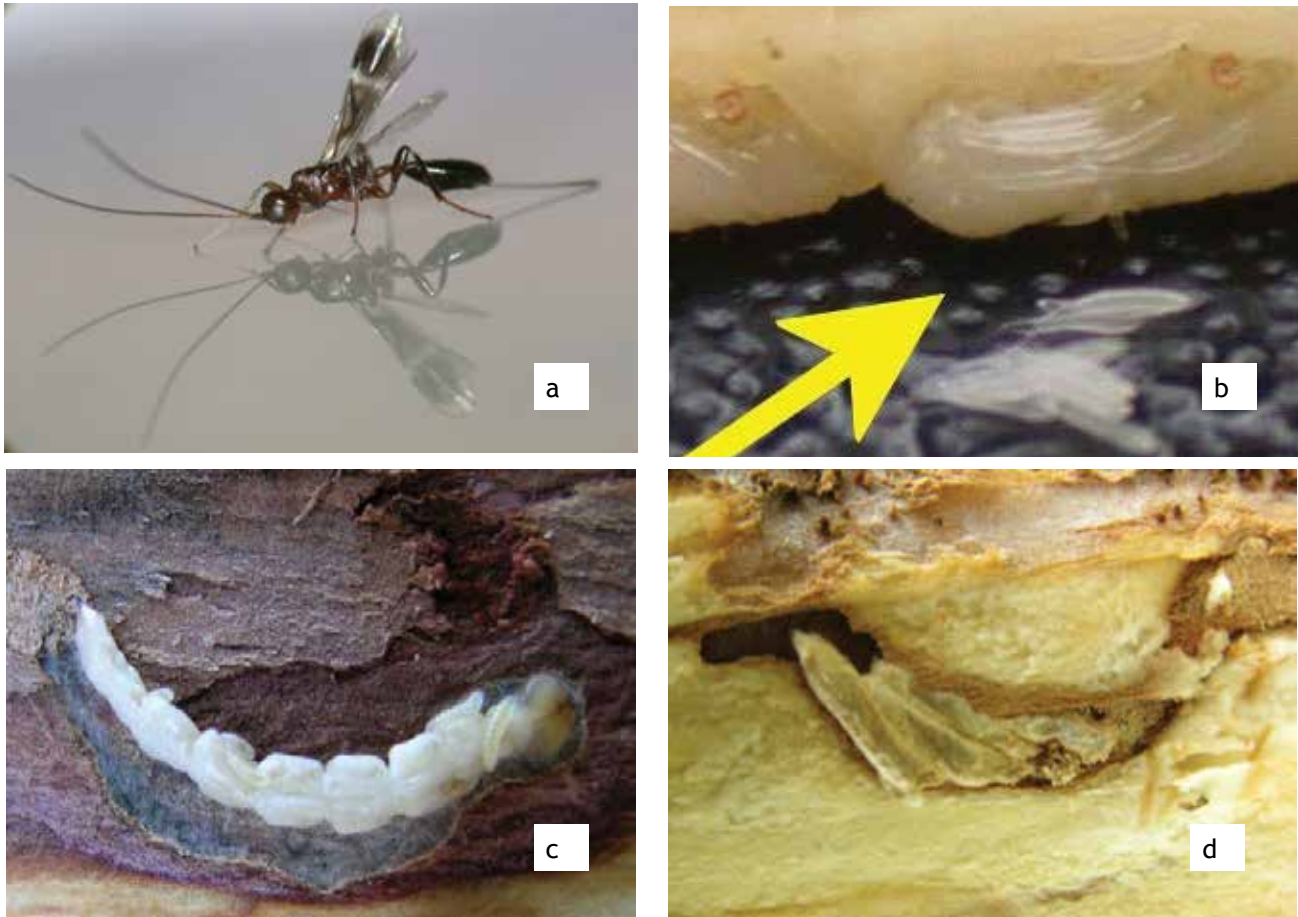
Figure 7. *Spathius agrili* life stages: (a) *S. agrili* adult female. (Photo credit: Tracy Ayers); (b) *S. agrili* eggs on an emerald ash borer larva. (Photo credit: Zhong-qi Yang); (c) *S. agrili* larvae feeding externally on an emerald ash borer larva. (Photo credit: Houping Liu); (d) *S. agrili* cocoons in emerald ash borer larval gallery. (Photo credit: Houping Liu)

Figure 8. *Spathius galinae* adult female ovipositing in an ash log. (Photo credit: Jian Duan)

United States and narrow host specificity, researchers requested permission to release *S. galinae* as an EAB biological control agent in the United States in 2014.

CONCLUSIONS

Although EAB is attacked by a diversity of native parasitoids of wood-boring beetles in North America, their prevalence is relatively low compared to that of the EAB-parasitoid complex in northeast Asia, where this buprestid originated. Consequently, classical biological control of EAB, with the introduction of three parasitoid species from China, was initiated in Michigan in 2007. By 2012, the establishment and increasing prevalence of two introduced parasitoids, *T. planipennisi* and *O. agrili*, was confirmed in Michigan and several other states. Besides increasing parasitism by the introduced and native parasitoid species, other important mortality factors are now known to suppress EAB population densities including host resistance in healthy ash trees, woodpecker predation, and entomopathogens. With the continued persistence of EAB and ash in the environment, we expect a cumulative effect of biotic and abiotic mortality factors to suppress EAB population densities below a tolerance threshold, ensuring the survival and reproduction of some native ash. The continuation and expansion of long-term field studies in EAB-infested forest ecosystems, where EAB biological control agents are released, is essential for further development of an integrated pest management approach to EAB in North America.

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CHAPTER 7: TRAPPING TECHNIQUES FOR EMERALD ASH BORER AND ITS INTRODUCED PARASITOIDS

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## SURVEY AND DETECTION OF EMERALD ASH BORER

As soon as emerald ash borer (*Agrilus planipennis* Fairmaire) (EAB) was discovered near Detroit, Michigan, USA, in 2002, surveys were initiated to delimit the extent of the infested area. These initial delimitation surveys were based on visual assessments using external symptoms because at the time no other detection tools were available and nothing was known about EAB responses to chemical or visual stimuli. Surveys were supplemented by tracing movement of nursery stock shipped from Detroit to other locations to detect new infestations of EAB. External symptoms of EAB infestation, which include D-shaped exit holes, dieback and crown thinning, epicormic shoots, and bark splits over galleries, are not apparent until one or more years after trees are infested by which time some adult beetle emergence may have occurred, allowing dispersal to other locations (Poland and McCullough, 2006). Therefore, visual surveys that rely on detecting infested trees are not effective for discovery of low-density infestations.

As of 2014, development of better detection tools for EAB remained an important need for the regulatory program. Research on EAB behavior demonstrated that adult beetles respond to volatiles emitted by stressed ash (Rodriguez-Saona et al., 2006) and preferentially oviposit on girdled trees (McCullough et al., 2009a,b). Based on this finding, in 2004 the Michigan Department of Agriculture implemented a statewide survey employing grids of girdled trap trees (Rauscher, 2006; Hunt, 2007). Large, open-grown ash trees were girdled in spring before EAB emergence by removing a band of bark and

phloem, approximately 16 cm wide, around the whole circumference of the tree. A band of plastic wrap, approximately 30 cm wide, was placed on the trunk above the girdle and coated with Tanglefoot insect trapping glue. Girdled trap trees were visually inspected during the summer to detect EAB adults on sticky bands; in fall or winter, girdled trees were felled and sections of the log were peeled to locate EAB larvae or galleries. Grids of over 10,000 trap trees were used for detection surveys in Michigan and several surrounding states up through 2008. While girdled trees are the most effective tool for detecting EAB (McCullough et al., 2011; Mercader et al., 2013), debarking trees to locate larval galleries is costly and labor-intensive, and suitable trees are not always available. Consequently, emphasis was placed on development of traps and lures that incorporated visual or olfactory cues to attract and capture EAB adults.

Odors from the leaves of stressed ash trees (Rodriguez-Saona et al., 2006), green leaf volatiles, especially *cis*-3-hexenol (de Groot et al., 2008; Grant et al., 2010, 2011; Poland et al., 2011), and sesquiterpene volatiles from ash bark elicit antennal responses and are attractive to EAB. Many of these attractive compounds are present in a natural tree oil called Manuka oil (Crook et al., 2008), and for this reason this oil was often incorporated into EAB traps.

Male and female EAB are sensitive to light in the ultraviolet (UV), violet, and green (420-430, 460, and 530-560 nm, respectively) ranges of the electromagnetic spectrum, while mated females are also sensitive to light in the red (640-670 nm) range (Crook et al., 2009, 2012). The beetles are attracted to green or purple traps hung in both the open and the ash canopy (Crook et al., 2009; Francese et al., 2010).
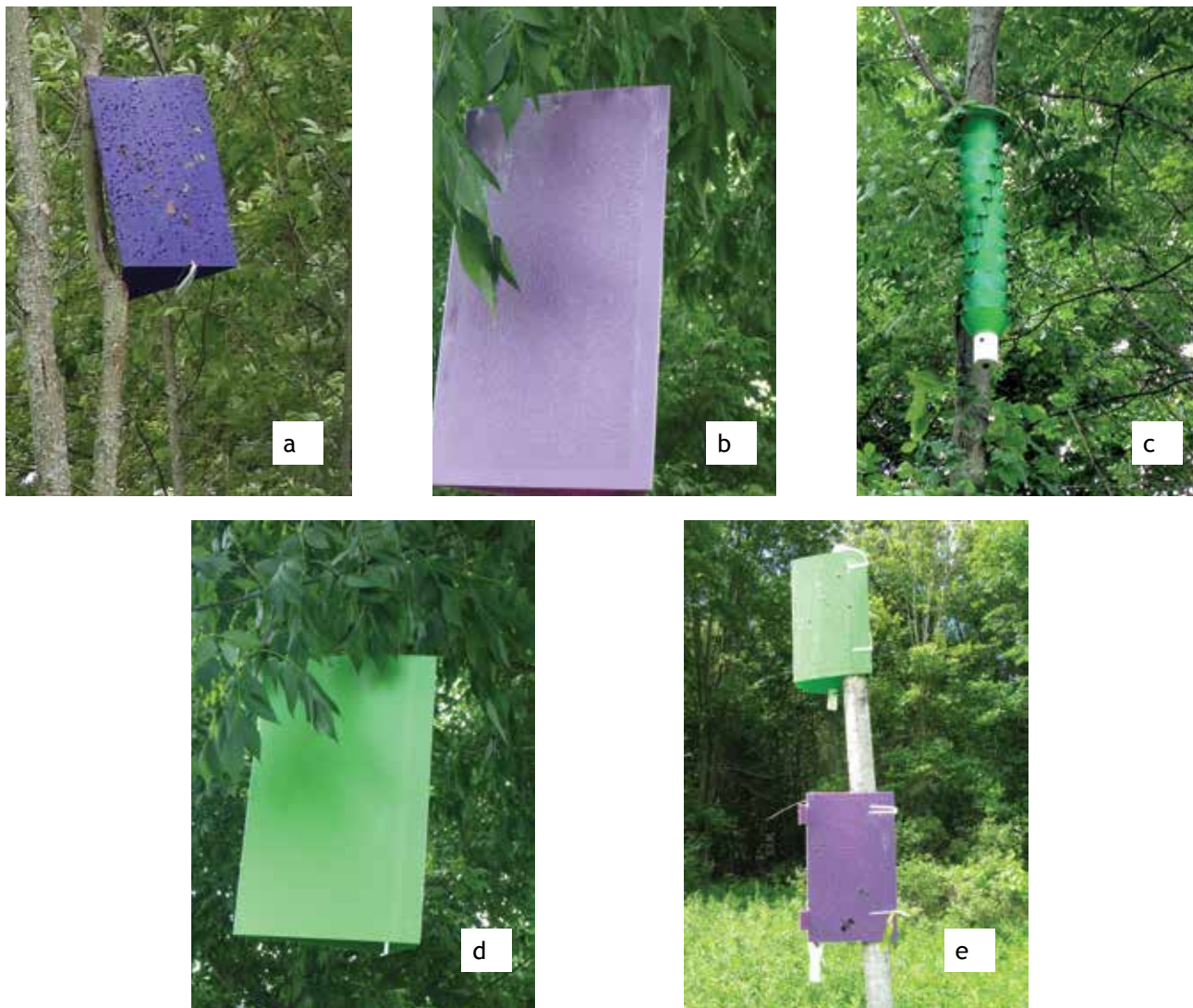
Figures 1 a-d. Various trap designs, colors and lure combinations suspended in the canopy of an ash tree: .(a) Dark purple sticky prism trap. (Photo credit: Therese Poland); (b) Light sabic purple sticky prism trap. Photo credit: Therese Poland); (c) Green multiple funnel trap coated with Fluon. (Photo credit: Toby Petrice); (d) Green sticky prism trap. (Photo credit: Therese Poland); (e) Green and purple double decker trap. (Photo credit: Therese Poland)

Males, which tend to hover near the canopy of ash trees (Rodriguez-Saona et al., 2007), are captured in higher proportions in green traps hung in the canopy of ash trees and baited with green leaf volatiles; in contrast, females, that oviposit on the trunks of ash trees are captured in higher proportions in purple traps hung below the canopy and baited with bark sesquiterpenes (Crook and Mastro, 2010; Grant et al., 2011). There is also evidence that close range or contact pheromones are involved in mate recognition and mating behavior (Lelito et al., 2009; Pureswaran and Poland, 2009) and that a female-produced volatile pheromone, *cis*-lactone, increases attraction of males

to green canopy traps baited with green leaf volatiles (Silk et al., 2009, 2011; Ryall et al., 2012).

Artificial traps were first used by USDA Animal Plant Health Inspection Service (APHIS) in a national EAB detection survey in 2008 (Crook and Mastro, 2010). Traps consisted of 3-sided prisms made of standard dark purple corrugated plastic (Coroplast Inc., Dallas, TX; 421 nm, 16.3% reflectance; 605 nm, 9.5%; 650 nm, 14.2%). Traps were coated with clear insect trapping glue, hung in the canopy of ash trees, and baited with Manuka oil lures with release rates of 50 mg/day (Synergy Semiochemicals, Burnaby, B.C.) (Fig. 1a). Various trap designs, colors, and lure

combinations were tested and detection surveys modified to incorporate the latest research findings. Starting in 2014, a new lighter shade of purple (Great Lakes IPM, Vestaburg, MI; Sabic purple, 413 nm, 32.8%; 613 nm, 18.8%; 650 nm, 28.5%) was employed for the sticky prism traps hung in the canopy of ash trees. Also, cis-3-hexenol lures releasing 50 mg/day (Scentry Biologicals, Inc., Billings, MT) have been added to the Manuka oil lures (USDA APHIS, 2014) (Fig. 1b).

Other promising traps under evaluation as of 2014 included (1) green (530 nm, 57% reflectance) multiple funnel traps (Chemtica Internacional, San Jose, Costa Rica) coated with Fluon, a slippery polymer (Northern Specialty Chemicals, Dudley, MA), and baited with cis-3-hexenol released at 50 mg/day (Scentry Biologicals, Inc., Billings, MT) (Francese et al., 2011) (Fig. 1c), (2) green (540 nm, 49% reflectance) sticky prism traps hung in the canopy of ash trees baited with cis-3-hexenol and the EAB pheromone cis-lactone (Sylvar Technologies, Inc., Fredericton, NB) (Ryall et al., 2012) (Fig. 1d), and (3) double decker traps made of a 10 foot PVC pole to which a green sticky prism (540 nm, 49% reflectance) is attached at the top and a light purple sticky prism (413 nm, 32.8%; 613 nm, 18.8%; 650 nm, 28.5%) is attached 60 cm below (Great Lakes IPM, Vestaburg, Michigan); both prisms are baited with two cis-3-hexenol bubble caps releasing 3.7 mg/day per bubble cap (ConTech Enterprises, Inc., Delta, B.C) (Poland et al., 2011, Poland and McCullough, 2014) (Fig. 1e).

The 2014 national emerald ash borer survey included (1) a nationwide survey of 8800 traps, set outside the 100 mile wide buffer zone surrounding the known infested area in locations at risk for introduction and establishment of EAB and (2) a leading edge survey employing 13,200 traps set within the 100 mile wide buffer zone. Traps were set within 1 km^2 cells that were selected using a risk-based model that incorporated risk factors that included proximity to campgrounds, major transportation arteries, truck stops, sawmills, firewood vendors, tree nurseries, recently landscaped properties, and high attendance cultural event sites. A trap was placed within each 1 km^2 cell, avoiding habitats of threatened or endangered species. Traps were placed in the

lower to mid-canopy of ash trees, preferably 20 cm or more in diameter, along edges or open areas on the sunny side of trees. The bottom edge of the trap was 150 cm or more above ground (USDA APHIS, 2014). In addition, any ash trees within each 1 km^2 cell exhibiting two or more symptoms of emerald ash borer infestation (dieback, epicormic shoots, bark splits, woodpecker damage, D-shaped exit holes, or visible serpentine galleries) were destructively sampled by removing bark to reveal emerald ash borer galleries and larvae.

Traps were placed in the field just before 250 growing degree days (base 10 °C) were accumulated, which corresponds approximately to the time when emerald ash borer emergence begins. Lures were replaced within 60 days. Traps were checked at a minimum when lures were replaced and when traps were taken down. All captured EAB and suspect beetles were collected and submitted to the State Plant Health Director or APHIS representative for species determination. Traps remained in place until after August 1 and 833 growing degree days (base 10 °C) had accumulated (USDA APHIS, 2014).

SURVEY AND DETECTION OF INTRODUCED EAB PARASITIOIDS

For parasitoids introduced for biological control, both their establishment and impact on the target pest must be measured. Establishment means the development of a successfully reproducing, self-sustaining population of the natural enemy, complete with overwintering survival for one or more years. Parasitoid establishment cannot be determined until at least one year after parasitoid release. Evaluating the impact of a natural enemy on the population of the target pest requires an estimate of the mortality caused by the natural enemy to the host; often this is equivalent to the generational rate of percentage parasitism in the naturally occurring host population in the field. Some parasitoids can kill hosts by means other than parasitoid reproduction, such as host feeding or stinging hosts without laying any eggs (DeBach, 1943; Van Driesche and Taub, 1983; Jervis and Kidd, 1986; Kidd and Jervis, 1989; Heimpel and Collier, 1996; Jervis et al., 1996); however, the

Figure 2 a,b. Laboratory produced emerald ash borer eggs on bark flakes placed under bark flaps cut into ash trees in the field. (Photo credit: Jian Duan)

parasitoids currently being released against emerald ash borer do not have these behaviors.

In the case of the emerald ash borer, there are two beetle life stages targeted by parasitoids: the egg and larva. The egg parasitoid, *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae) (Zhang et al., 2005) and two larval parasitoids, *Tetrastichus planipennis* Yang (Eulophidae) (Yang et al., 2006) and *Spathius agrili* Yang (Hymenoptera: Braconidae) (Yang et al., 2005), have been imported and released in North America (Bauer et al., 2008). Various methods have been developed to assess the establishment and impact of these parasitoids and they are reviewed in this chapter.

EGG PARASITOIDS

Three approaches have been used to detect establishment or measure the impact of the egg parasitoid *O. agrili*: (1) deploying laboratory-produced host eggs in the field as sentinel eggs, (2) using yellow pan traps to passively collect *O. agrili* adults, and (3) collecting wild (naturally occurring) emerald ash borer eggs in the field.

Sentinel Eggs

Deployment of sentinel eggs can detect the presence of *O. agrili* at particular sites, which, if appropriately timed, can indicate establishment. Several methods have been developed for field-deployment of emerald ash borer eggs produced in the laboratory. The first

of these involves cutting a small flap of bark on an ash tree and placing EAB eggs under this flap (Fig. 2). Eggs can be collected later and examined for parasitism. This method, however, suffers from a high degree of egg predation (Duan et al., 2011). The second method is based on the field-deployment of ash logs bearing laboratory-laid EAB eggs. These egg sentinel logs (ESL) are made by wrapping curling ribbon around a bolt of ash (ca 5 cm in dia by 25 cm long) and placing them in a container with gravid EAB for several days (Fig. 3). The tight space between the curling ribbon and the ash bolt stimulates EAB oviposition (Fig. 4) and partially conceals the egg from predators in the field. A more detailed description of ESL production can be found in Duan et al. (2012a) and USDA APHIS/ARS/FS (2013). Once produced, ESL units can be hung on or near ash trees and left for several weeks. It should be noted, however, that depending on temperature EAB eggs are only suitable for parasitism up to the development of the neonate host larva (approximately 8-10 days after oviposition). Once collected from the field, eggs on ESL units can be held in the laboratory to rear *O. agrili* adults. Alternatively, each egg can be inspected under a microscope for visual signs of parasitism (Fig. 5). A third method of deploying sentinel eggs is to place host eggs inside various protective enclosures, such as plastic cups (with or without a screened opening) or pouches made entirely of screening. Screening is used to exclude predators while allowing access to eggs by *O. agrili*. While field recoveries of *O. agrili* have been made using this method, it

Figure 3. Egg sentinel log with curling ribbon in container with emerald ash borer. (Photo credit: Deborah Miller)

Figure 4. Egg sentinel log with curling ribbon removed showing EAB eggs. Black eggs are parasitized and brown eggs are unparasitized. (Photo credt: Kristopher Abell)

Figure 5. (a) Parasitized EAB egg with parasitoid emergence hole and typical black coloration. (b) Parasitized EAB egg with meconium inside visible due to atypical brown coloration. (Photo credit: Deborah Miller)

generally seems less effective compared to use of ESL units. Currently, therefore, of the three methods used to deploy emerald ash borer eggs in the field to detect egg parasitism, use of sentinel egg logs is the preferred method (Fig. 6).

Yellow Pan Traps

Yellow pan traps are yellow plastic bowls (Fig. 7)

mounted to ash trees with a shelf bracket nailed to the tree. A second bowl can be placed inside the mounted bowl to allow for easy removal and processing of the sample. This second bowl is filled with a 20% clear propylene glycol solution and a drop of unscented detergent. Yellow bowls are used because this color is generally attractive to many parasitoids, and detergent decreases the surface tension of the water, causing most insects to sink and drown. Trap contents

Figure 6. Egg sentinel log suspended from an ash tree in the field. (Photo credit: Kristopher Abell)

Figure 7. Yellow pan trap. (Photo credit: Leah Bauer)

Figure 8 a,b. Emerald ash borer eggs on ash bark in the field. (Photo credit: Jian Duan)

Figure 9. Removal and collection of outer ash bark with a drawknife for assessment of *Oobius agrili* using emergence tubes and bark sifting. (Photo credit: Kristopher Abell)
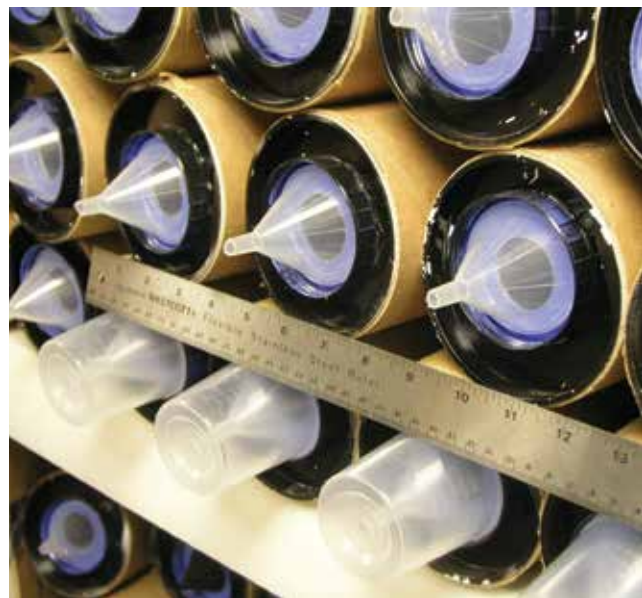
Figure 10. Emergence tubes used to collect emerging *Oobius agrili* from bark samples. (Photo credit: Deborah Miller)

should be collected after several days depending on temperature to avoid rotting. Once collected, the contents of the pan trap can be examined for the presence of *O. agrili* adults (JG, unpublished data). A detailed step-by-step guide to the construction and setup of yellow pan traps can be found in "Emerald Ash Borer Biological Control Release and Recovery Guidelines" (USDA APHIS/ARS/FS, 2013). Yellow pan traps are non-selective and may not detect low density populations of *O. agrili*.

Naturally Occurring EAB Eggs

Assessing the impact of *O. agrili* (i.e., percent parasitism for EAB populations) is a more difficult task than determining if *O. agrili* is established at a site. To assess the impact of *O. agrili* on naturally occurring EAB egg populations, EAB eggs must be collected in the field. Finding EAB eggs in the field is difficult because adults lay their eggs between layers of bark or in bark fissures on ash trees (Fig. 8). Two methods have been developed to collect wild EAB eggs. The first collection process is a timed visual inspection of ash bark, using a utility knife to parse away bark layers. An arbitrary but fixed amount of time (generally 30 minutes) is spent searching each tree to maintain consistent sampling effort. Eggs found are returned to the laboratory to be inspected with a dissecting microscope for signs of parasitism (Fig. 5) (Duan et al., 2011, 2012a).

A second method to measure rates of parasitism in wild EAB eggs is based on the physical removal of the outer bark of ash trees over a fixed area, inside of which layers of bark are scraped off using a drawknife (Fig. 9). If assessing establishment is the only goal, then sampled bark can be placed in emergence tubes (Fig. 10) and monitored for *O. agrili* emergence. Emergence tubes are typically made from cardboard mailing tubes, but other light-excluding containers can be used. One end of the tube is sealed against light while an inverted funnel and translucent collection cup (Fig. 11) is mounted on the other end. Tubes should be held in a well lit environment at 18-32 °C. At low densities, *O. agrili* may be difficult to detect because the parasitoids do not always find their way out of the emergence tube and into the collection cup. A more reliable way to detect *O. agrili* in bark

Figure 11. Close-up view of *Oobius agrili* emergence tube, cup and funnel. (Photo credit: Deborah Miller)

Figure 12. Sifting bark samples using standard nylon window screening. (Photo credit: Deborah Miller)

samples, which also allows for assessment of impact, is to examine the collected bark. While a complete search of the entire bark sample would be the most effective, it takes too long. Therefore, a subsampling approach was developed that involves sifting the bark sample with standard nylon window screening and determining rates of parasitism in eggs that pass through the screen. The bark sample is placed on window screening and shaken for three minutes (Fig. 12). Many eggs are dislodged while shaking and fall through the openings in the screening along with small bits of bark debris. The material that passes through the sieve is then examined for EAB eggs using a microscope, and each egg is evaluated for parasitism. An estimate of percent parasitism can be obtained from each of these methods (timed visual search and bark sifting) by dividing the number

of parasitized eggs by the total number of eggs (parasitized and not, both emerged and not emerged, live and dead).

Advantages and Disadvantages of Methods for Detection of Egg Parasitoids

The use of sentinel eggs, whether under bark flaps, on ESL units, or in protective enclosures, is subject to predation. Very often predators may remove most, or even all, sentinel eggs. The presence of curling ribbon on the logs used in the ESL units and screening over protective enclosures around the ESL unit both reduce but do not eliminate predation. Additionally, a substantial amount of infrastructure and manpower is needed to maintain an EAB colony, which is required to produce eggs for field deployment. When creating ESL units, a sufficient number of eggs (~50-100) must be produced per log and deployed in the field within 2-3 days. Older eggs are not preferred for parasitoid oviposition and their deployment produces little useful data. Time of deployment of sentinel eggs must also be carefully considered to coincide with seasonal occurrence of *O. agrili* adults. Deployment of sentinel eggs too early or too late in the year would result in false negatives when assessing establishment. In Michigan, *O. agrili* adult females first appear after approximately 445-556 degree days (base 10 °C) (Abell, unpublished data).

Yellow pan traps may be a relatively easy method to assess establishment of *O. agrili*. Unlike methods using sentinel eggs, pan traps do not require the maintenance of an EAB colony to produce eggs and the time constraints associated with egg viability are not an issue. Furthermore, since pan traps have the potential to also catch larval parasitoids of EAB this may increase their utility. However, there are several important disadvantages to consider. First, the incidental trap-catch of other similar-looking hymenoptera or other insects can be substantial. When such incidental catch is high, more time is required to examine and sort through the sample, which is particularly difficult considering the small size of *O. agrili*. Because of its small size, *O. agrili* can often become entangled in the setae of other insects making them easy to miss. Second, the effectiveness of yellow pan traps is largely unknown. Some work has shown

pan traps to be more effective than ESL units and other sentinel egg methods, while other work has shown the opposite (Parisio, unpublished data; Bauer et al., 2011a).

Timed visual egg surveys and bark sifting allow for assessment of establishment and estimation of percent parasitism of naturally occurring field populations, but each has disadvantages to consider. Both methods collect EAB eggs from several generations and there is no way to differentiate old eggs from new ones. Because of this it can be difficult to assess year-to-year fluctuations in rates of parasitism at a site. Consideration of aspect (cardinal direction) of the sampling point on the tree is also important for each method. Sampling from only one side of a tree may introduce a bias. In general, sampling around the full circumference of the tree is recommended for estimating field rates of parasitism. However to only estimate establishment, egg density and parasitism rates are greatest on the south and west sides of tree (Abell et al., 2014). Visual egg surveys have several special disadvantages: finding eggs on standing ash trees in the field is difficult even with the aid of a magnifying lens, searching is affected by light and weather conditions in the field, and the process of removing small bits of bark while searching likely results in the loss of some eggs along with the removed bark. The bark sifting method does not have these disadvantages, but is more time consuming.

Regardless of the method used, *O. agrili* is particularly challenging to sample. Work done using all the above methods has shown that, at least during the first several years following the species release at a site, *O. agrili* has a very patchy distribution. Often, only 10-20% of trees sampled within several hundred meters of each other will result in recovery of *O. agrili* (Abell et al., 2011). Therefore a large number of trees (>10) need to be sampled to adequately assess *O. agrili* levels.

LARVAL PARASITOIDS

Several methods have been used to detect establishment and assess impact of larval parasitoids of emerald ash borer: (1) deployment of laboratory-reared EAB larvae in the field as sentinels, (2) using yellow pan traps to passively collect adult parasitoids,

and (3) collecting naturally occurring EAB larvae in the field for dissection or rearing.

Sentinel Larvae

There are several methods that employ sentinel larvae to assess establishment of parasitoids of emerald ash borer larvae. Larval sentinel logs (LSL) are similar in concept to the ESL units described previously for detection of the egg parasitoid. To construct LSL units, third to fourth instar EAB larvae are inserted into ash bolts approximately 5 cm in diameter and 25 cm long. Bolts are sealed on both ends with paraffin wax to prevent desiccation. To insert larvae, a portion of inner bark and wood approximately the same size as an EAB larva is excavated from the log, and an EAB larva is placed in the grove and covered by the remaining flap of outer bark (Fig. 13). Several EAB larvae can be inserted into a bolt in this manner. Then, after the outer bark flaps are secured, the area of the log where a larva has been inserted is further protected by wrapping it with parafilm. Care should be taken to sterilize the tools used to create excavations and handle larvae to avoid introducing pathogens. LSL units are then placed on ash trees in the field (Fig. 14) and left in place for 1-2 weeks. How long LSL units last in the field depends upon the age of EAB larvae and the temperature. Since EAB larvae tunnel into the heartwood of ash to pupate, they become inaccessible to parasitoids at that point. Temperature affects the rate of development of EAB larvae; also higher temperature increases desiccation of LSL units. LSL units can also be produced by placing emerald ash borer eggs on ash bolts and allowing newly hatched larvae to bore into bolts; bolts are then held at a constant temperature until larvae reach the appropriate instar. This second method, however, is less desirable because the number of EAB larvae in each bolt will be unknown since some eggs won't hatch and some larvae will die. In addition, LSL units produced in this manner seem to be less effective at detecting parasitism, possibly because cuts made when inserting larvae emit volatiles that attract parasitoids (Abell, unpub.). These two methods – inserting larvae or affixing eggs to bark – can also be applied to live ash trees in the field (Ulyshen et al., 2010; Abell et al., 2012). Additionally, adult EAB can

Figure 13. EAB larvae placed in excavated area of an ash bolt to create a larval sentinel log. (Photo credit: Kristopher Abell)

Figure 14. Larval sentinel log hung on an ash tree in the field. (Photo credit: Kristopher Abell)

be caged directly onto the trunk of live ash trees and allowed to oviposit eggs (Duan et al., 2014).

Yellow Pan Traps

The setup, advantages, and disadvantages of pan traps to capture EAB larval parasitoids are much the same as when they are used to detect egg parasitoids, as described above. As stated above, yellow pan traps are non-selective and may not detect low-density

populations of released EAB larval parasitoids. Recently the pheromones of *Spathius agrili*, *Spathius floridanus* Ashmead, and *T. planipennisi* have been identified (Bauer, et al., 2011b, Cossé et al., 2012), and these materials can be used as attractants in combination with yellow pan traps to increase trapping efficiency.

The pheromones for the two *Spathius* species are male-produced aggregation pheromones attracting both male and female insects. The pheromone for *T. planipennisi* is a female-produced sex pheromone attracting males.

The attractiveness of synthetic *S. agrili* pheromone was tested in a large (3.7 x 6.1 x 3.7 m) outdoor field cage using eight (1.8 m high) evenly spaced potted evergreen ash (*Fraxinus uhdei* [Wenz.] Lingelsh.) plants. Yellow sticky board strips (Fig. 15) were placed in each plant halfway up. The pheromone was impregnated into rubber septa, affixed to the sticky boards. Approximately 45% of the released males and 50% of the released females were recaptured on the pheromone-baited traps during the 24 h trapping periods (Fig. 16) (Cossé et al., 2012), compared to 10% of released males and 5% of released females for yellow traps without pheromones. Field trapping of *S. agrili* using yellow pan traps and pheromone has not yet been demonstrated due to a lack of established populations of *S. agrili*.

For *T. planipennisi*, wind tunnel behavioral studies have demonstrated that male *T. planipennis* are highly sensitive to a female-produced pheromone with optimal responses to pheromone at 20 pg/µl. Under summer conditions, this dosage of pheromone is likely to be attractive for *T. planipennisi* males for at least two weeks. A field test was run in August-September, 2013 in East Lansing, Michigan where *T. planipennisi* has an established population. Twenty yellow pan traps were deployed following the method described, ten with and ten without pheromone lures. Septa were replaced by fresh ones after two weeks. Of 40 males trapped, 39 were captured by pheromone-supplemented traps, while control traps (yellow only) caught one parasitoid (Fig. 17).

The above results demonstrate that EAB parasitoid pheromones can increase efficiency of yellow pan traps. Pheromones of *Spathius* sp. and *T. planipennisi*

Figure 15. Field cage setup for release and recapture of male and female *Spathius agrili* with yellow sticky traps baited with parasitoid pheromone. (Photo credit: Allard Cossé)

are stable under field conditions and only small amounts of the pheromones are needed to attract the target parasitoids. A disadvantage of using pheromones is that they will have to be synthesized, since the compounds are not commercially available.

Naturally Occurring EAB Larvae

Sampling naturally occurring EAB larvae is the only way to estimate percent parasitism by larval parasitoids. To collect EAB larvae, the bark of living EAB infested ash trees is peeled off, usually with a drawknife (Fig. 18). Larvae can then be examined in the field or taken back to the laboratory to be dissected or reared to detect parasitoids (see Chapter 6 for pictures and descriptions of parasitoid life stages). Several studies have demonstrated the effectiveness of this method (Duan et al., 2012b, 2013a,b, 2014).

Advantages and Disadvantages of Methods for Detection of Larval Parasitoids

Similar to the use of sentinel eggs, deployment of sentinel larvae requires substantial infrastructure and manpower. EAB must be reared from the egg to 3rd or early 4th instar larval stage to be suitable for use. Predation of sentinel larvae is not a problem, but bacterial or fungal contamination can be, and once introduced into colonies, pathogens can become pervasive and difficult to eliminate. Additionally, un-infested ash is needed both for rearing EAB larvae and creating LSL units. Finding un-infested ash of the
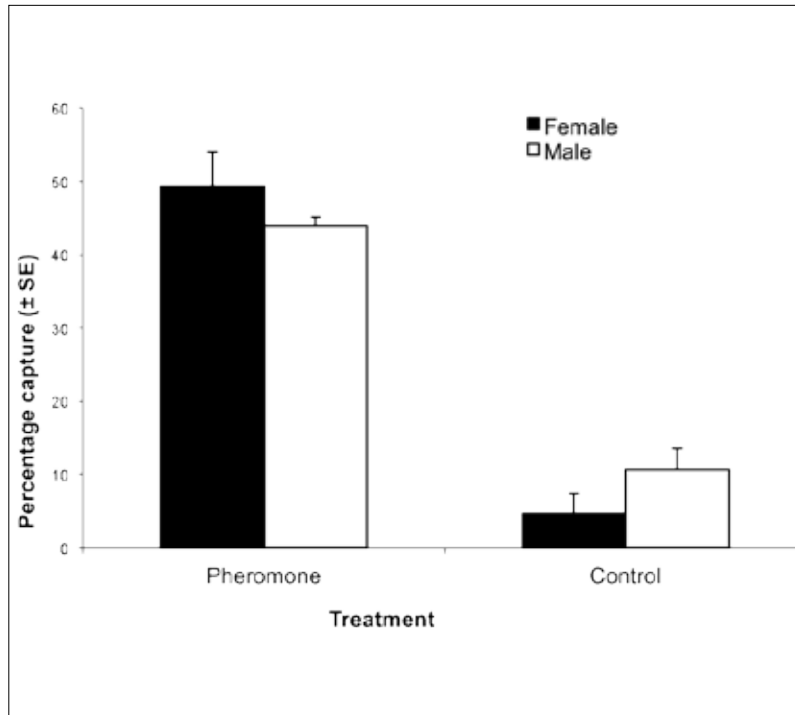
Figure 16. Percentage (± SE) of captured virgin male and female *Spathius agrili* on yellow sticky traps baited with pheromone.
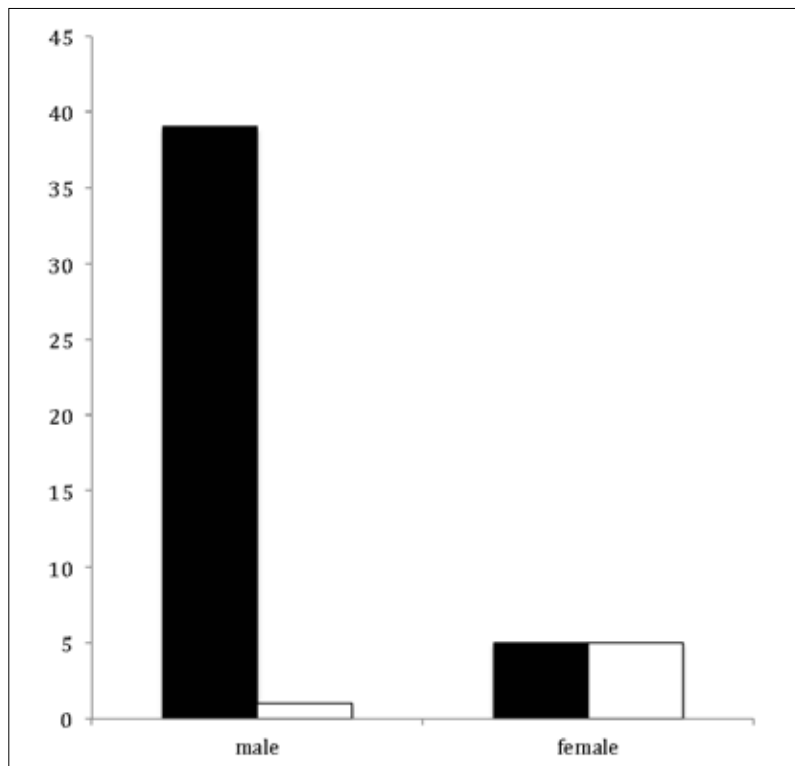
Figure 17. Total number of trapped male and female *Tetrastichus planipennisi* on yellow pan traps baited with pheromone.

Figure 18. Peeling bark from ash trees to search for naturally occurring emerald ash borer larvae. (Photo credit: Leah Bauer)

appropriate size can be difficult, especially in regions where EAB is abundant. Also, native parasitoids that attack EAB, such as *Atanycolus* spp. (Hymenoptera: Braconidae), sometimes attack many of the sentinel larvae, thus preventing assessment of introduced parasitoids. Despite these disadvantages, sentinel larvae allow for standardized, nondestructive detection of larval parasitoids. In addition, sentinel larvae can be deployed at any time during the field season. It is important, however, to deploy them when larval parasitoids are likely to be present (May-September).

Sampling naturally occurring EAB larvae by peeling the bark of infested trees eliminates the need to rear and maintain EAB larvae, which must be done for sentinel larval methods. It also allows for the direct assessment of what is currently occurring in the field and estimation of attack rates by larval parasitoids. Bark peeling is destructive, however, so unlike the use of sentinel larval methods, in plots where there is a need for repeated sampling, collection of wild larvae must be limited to preserve trees for future work. Peeling bark is laborious and requires careful technique to avoid damaging larvae, since damaged larvae are often difficult to diagnose for parasitism, particularly

for ectoparasites like *Spathius* spp. and *Atanycolus* spp., which can easily be dislodged and lost. In addition, cases in which larval parasitoids have already emerged are often difficult to diagnose. Finally, woodpecker predation can be high (Lindell et al., 2008; Duan et al., 2012b, 2013a, 2014; Jennings et al., 2013), and it is not possible to determine if larvae taken by woodpeckers were also parasitized or not.

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CHAPTER 8: MASS-REARING OF EMERALD ASH BORER AND ITS PARASITOIDS

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INTRODUCTION

Mass rearing of emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire) (Coleoptera: Buprestidae) poses significant challenges both in terms of understanding its biology and phenology in the field and maintaining sources of insect material in the laboratory. Fortunately, in the past few years, significant progress has been made by several USDA facilities on optimizing laboratory rearing of emerald ash borer for a variety of purposes (Duan et al., 2013ab).

Rearing the natural enemies of wood boring insects is difficult because of the need for effective means to mass rear the host, which has a long (one or two year) life cycle and cryptic life history. At the initiation of any biological control program for a newly detected invasive wood boring pest such as EAB, information on the target pest as well as its natural enemies may be very limited. Using the emerald ash borer/parasitoid system as an example, the three primary natural enemies, now being produced and released across the United States, were newly described in the first several years following an intensive foreign exploration program (USDA APHIS, 2007) in response to the detection of the borer by federal agencies.

At their simplest, the critical resources required for mass production of natural enemies of emerald ash borer are growth chambers with good temperature, humidity, and photoperiod control, fresh ash foliage (preferably produced in the field), and small-diameter, clean ash logs from sapling trees (either from the field or grown in the greenhouse). An adequate supply of EAB larvae or adults can sometimes be harvested from the field for laboratory use from nearby EAB infestations, but laboratory rearing is recommended to minimize disease and maximize beetle fecundity. Below, we discuss the current best-

practices for mass rearing the emerald ash borer and its parasitoids. It should be noted that as new parasitoids are introduced, their rearing may also be broadly similar to the methods presented here.

MASS REARNG EMERALD ASH BORER

Rearing emerald ash borer in any life stage is time consuming. Foliage provided to adult beetles must be replaced at least every four days; an efficient system for doing so is to maintain two sub-colonies, in which insects are given fresh foliage either on Monday and Thursday or on Tuesday and Friday. This step requires providing the insects with fresh foliage in a clean water vial in a fresh, clean container. No additional water is necessary, although misting the foliage during the provisioning process preserves foliage health.

Figure 1. Sex determination of emerald ash borer beetles. Female is on left; note larger size and wider abdomen, especially the two segments just posterior to the hindmost pair of legs. Males also have a pronounced silvery pubescence (the "beard") on the ventral surface of the anterior thoracic segments. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

Table 1. Effect of number of females per cage on emerald ash borer fecundity and longevity.

Number of Females at Initiation	% Enclosures w/Eggs	% Producing at 21 d	Mean Total Eggs	Mean Longevity (d)
1	56.8	74.6	156	31
2	70.2	78.0	190	37
3	82.0	58.7	96	40

Optimal rearing of adult beetles, to maximize laboratory production of eggs, can be achieved using two females and two males (see Fig. 1 for sex determination) provisioned with foliage as soon as possible following adult eclosion, although a single female per cage is also useful for some experimental needs (Table 1). Higher numbers of insects per cage is not recommended. At least two males should be used, regardless of the number of females, to ensure mating success (Rutledge and Keena, 2012). Beetles are allowed to emerge from field-collected ash logs in large cardboard barrels with funnels and jars at one end, and the beetles are collected in jars, to which beetles are drawn by light (Fig. 2). Alternatively, beetles can be reared through their entire life cycle in the laboratory in small ash logs (see below). Ash leaves used to feed adult beetles must be clean and free of pest damage (any decrease in nutrient content or increase in plant defense compounds will reduce EAB fecundity). The species of ash used as the source of foliage has significant effects on fecundity, with field-collected foliage of mature green ash (*Fraxinus pennsylvanica* Marshall) being the best for mass-rearing. During the winter, dark green, mature foliage from greenhouse-grown tropical ash (*Fraxinus uhdei* [Wenz.] Lingelsh.) can be substituted for green ash, but is generally inferior in quality to green ash and results in lower beetle fecundity. The EAB Rearing Facility in Brighton, Michigan (USA) has overcome this problem by having fresh young *F. uhdei* foliage shipped from southern California, where it grows as an ornamental. While this source of foliage is a more suitable than that from greenhouse-grown trees, shipping is expensive when considered over the course of a year.

To house the adult beetle colony, the EAB Rearing Facility uses 946 mL clear plastic cups and ventilated mesh lids (Fig. 3) such as those available from the following source: http://www.joshsfrogs.com/32-oz-insect-cup-and-lid-placon-cup-250-pack.html.

Figure 2. Cardboard rearing barrels, with funnels and vials inserted into the lids, into which emerald ash borer adults collect following their emergence from logs inside barrels. This is an efficient system for rearing adult beetles from field-harvested beetle-infested logs. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

Figure 3. Emerald ash borer enclosure and setup showing 946 mL plastic cup, Velcro, water vial with drilled lid (right), and ash foliage added (left). This is an appropriate amount of ash foliage for 3-4 insects for 3 days. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

Figure 4. Emerald ash borer rearing enclosures showing screen and filter paper lids secured for oviposition. Tracking egg production data is useful for optimizing colony demography. A simple data sheet, visible on side of cup, records the day of beetle emergence, weekly egg production, and the number of beetles dead at each provisioning with new foliage. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

Figure 5. Filter paper removed from the top of an emerald ash borer-rearing enclosure, showing eggs (brown dots). Number of eggs shown is typical production from two emerald ash borer females for 3-4 days. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

Inside this plastic cup, a small 5-6 cm diameter disk of paper towel is placed on the bottom to absorb moisture from frass and foliage. A 3-4 cm strip of Velcro (Sticky Back Velcro Tape, 8 m x 1.9 cm, www.uline.com) is applied to the inner surface of the plastic cup. The matching portion of Velcro is fixed to the cap of a 20 mL plastic scintillation vial (Fisher Scientific, Product # 03-341-72A). Several 5-7 mm diameter holes are drilled into the vial's cap. The vial thus serves as a removable water reservoir to keep ash foliage alive while the beetles feed (Fig. 3). Vials, fiberglass screens, ventilated mesh lids, and plastic cups are bleached after each use. Paper toweling and filter paper lids are replaced each time foliage is changed. EAB-rearing cages are kept in a walk-in growth chamber held at 27 (± 1° C) during the day and 22 (± 1° C) at night, with a 16:8 light-dark cycle. Relative humidity is held at 75-80 (± 5%). Groups of 3-4 EAB adults are housed this way for two weeks, at which time the type of lid used is changed to facilitate egg-laying and collection. The cage construction remains the same, but instead of a ventilated lid, a 10 x 10 cm square of black fiberglass window screen is placed on top of the plastic cup. On top of this, a single coffee filter paper (Meijer Brand, 8-12 cup size) is placed, and both are tightly secured against the plastic cup with several small rubber bands. EAB

adults perceive the fiberglass screening as a rough surface under which to deposit eggs (Fig. 4); eggs thus laid adhere to the easily removable filter paper, which can then be used either to rear egg parasitoids or EAB larvae (Fig. 5).

Egg production and EAB health should be closely monitored to ensure rearing success (data sheets on the front of cages assist in this effort; Fig. 4). Any insects that die should be promptly removed from their cages, and any cage that loses more than two of the initial four insects should be discarded. Any evidence of fungal infection, such as sporulating cadavers, must be dealt with swiftly, as outbreaks can quickly devastate a large colony. The best methods to limit infection and outbreaks are to thoroughly clean all supplies, inspect rearing cages before opening to prevent transfer of pathogens (e.g., "infected" cages are discarded unopened), and remove dead insects promptly. Any beetles showing reduced fecundity should immediately be isolated. Any rearing cage that fails to produce eggs by day 21 post-adult EAB eclosion should be discarded even if the beetles remain alive – it is likely these insects are of poor quality or diseased, as the majority of healthy EAB females will lay eggs by this time (Table 1). Egg production naturally declines after 9-10 weeks of adult life, and to maintain efficiency and limit disease

Figure 6. An ash log artificially infested with emerald ash borer eggs. Small pieces of filter paper bearing beetle eggs are secured to the logs, eggs facing inward to facilitate establishment of young larvae. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

Figure 7. Emerald ash borer larvae can be reared in freshly cut green ash logs (14 cm tall by 5-8 cm wide) held in plastic trays (25 x 30 cm) filled with 1-2 cm of clean water. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

all rearing groups should be discarded after this age or as soon as egg production begins to decline.

EAB eggs deposited on coffee filters (Fig. 5) provide a convenient means to transfer eggs to ash logs, for the production of EAB larvae and pupae. EAB eggs can be gently secured to ash logs (Fig. 6) using a strip of Parafilm (Fisher Scientific, Cat. No. S37440), and inoculated logs then allowed to develop in trays of clean water for several weeks (Fig. 7). The EAB Rearing Facility uses photographic developing trays that are available commercially (Cescolite, Item # CL1114T) to maintain logs at this stage. Trays of this type are advantageous because excess water can be easily poured out, and trays hold a large number of ash logs and are highly resistant to chemicals, allowing trays to be easily sterilized with bleach after use. Larvae serve as hosts for the larval parasitoids (see below) or can be allowed to mature and excavate pre-pupal cells, after which time they can be used for the production of EAB adults. Temperature has a significant effect on EAB development and rearing temperatures should generally be at or below 30°C for optimal development (Duan et al., 2013a), especially for adult beetles. A key exception may be the rearing EAB larvae at slightly higher temperatures (i.e., 32-33° C) for more rapid production of larvae to serve as hosts for the larval parasitoids (see below). At this temperature, mature, 4th instar larvae can be produced in about three weeks.

Production of EAB adults using the method described above for rearing larvae is advantageous, although time consuming. Where wild-collected material is available, trees infested with numerous EAB can be felled and later warmed for beetle emergence in the laboratory. Field-collected material is only available seasonally, however, and long-term storage (e.g., from winter of the current year until the next autumn or winter) severely decreases the quality of the insects produced. To avoid these problems, EAB-infested ash logs can be incubated at warm temperatures for several months to allow full larval development, then chilled for several months, and later warmed for the production of EAB adults. Laboratory-reared adults suffer lower incidence of disease and generally have much higher fecundity than field-collected EAB adults because the duration of cold storage can be precisely controlled.

MASS REARING EAB PARASITOIDS

Oobius agrili

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae), a solitary egg parasitoid of EAB, can easily be reared with some modifications of the methodology developed by Liu and Bauer (2007). All laboratory colonies are parthenogenetic, and each female is capable of successfully parasitizing

at least several dozen EAB eggs in her lifetime (Liu and Bauer, 2007). An efficient mass-rearing system has been developed using a two-generation system: post-diapause *O. agrili* rearing cages are kept in a walk-in growth chamber at 25 (± 1° C), with a 16:8 light-dark cycle. When provisioned with EAB eggs, these insects will produce progeny, a majority of which emerge within three to four weeks. Some diapausing progeny will be produced under long-day conditions (generally around 20% of the total) and can be separated by examination under a microscope after non-diapausing progeny are allowed to emerge, by collecting those parasitized eggs that have no exit hole. It is important to note that this process is extremely time-consuming in a mass-rearing setting, where more than ten thousand EAB eggs must be examined per week, and is recommended only if all progeny must be collected for specific experimental needs. Non-post-diapause *O. agrili*, produced as outlined above, are transferred on the day of eclosion to rearing cages kept in a walk-in growth chamber held at 25 (±1° C), with an 8:16 light-dark cycle. Relative humidity under both photoperiod regimes is held at a constant 75-80 (± 5%). The non-post-diapause individuals, reared under short-day conditions, will produce diapausing progeny, which can then be stored for up to ten months at 4 ± 1° C and high (>75-80%) relative humidity. Storing *O. agrili* in diapause can be done as follows: 21 days after first exposure of fresh EAB eggs to adult *O. agrili*, the parasitized eggs are transferred into clean cups (i.e., no honey) and moved from 25° C to 10° C. After one week at 10° C, parasitized eggs are transferred to 4° C until needed. Post-diapause adults begin to emerge from cold-stored material after about one month of being returned to 25° C, and the cycle can be repeated.

Oobius agrili adults are very small and can easily crawl through very small openings, including all types of screen tested thus far (even insect netting), so care must be taken to maintain proper housing or adults will readily escape. *Oobius agrili* wasps can be securely housed in clear plastic 473 mL cups (Gordon Food Service, Item # 7922500) fitted with very tight, solid clear-plastic lids (Solo Brand, Item # 626TP-0090). Lids can be re-used, but must be assessed for
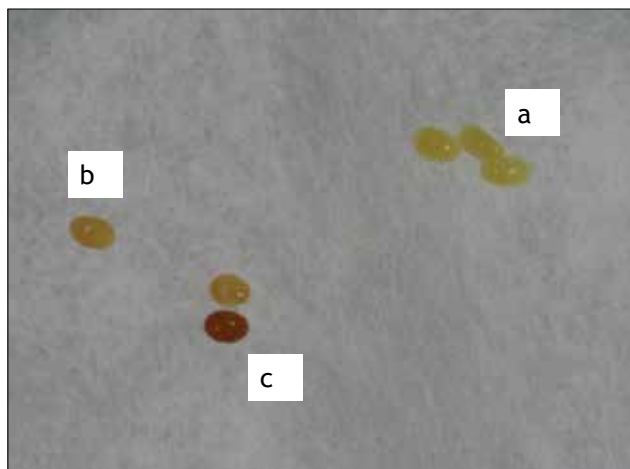
Figure 8. Emerald ash borer eggs at various stages of development. (a) freshly laid, suitable for oviposition of *Oobius agrili*; (b) 24-36 hours old, suitable for oviposition by *Oobius agrili*; (c) upper egg deflated and perhaps damaged; lower egg 2-3 days old and less likely to be parasitized unless presented immediately. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

tight fit – if the lid is used many times, it can become deformed and this may allow insects to escape. Nutrition must be provided to the wasps to ensure a normal life span of 2-3 weeks and optimal progeny production. Honey can be streaked directly onto the interior walls of the plastic cup using a very fine tool, such as a single hair from a brush. Care must be taken to ensure that the streaks are fine (<0.25 mm) so that the wasps do not become trapped as they attempt to feed. The relative humidity in the rearing environment will cause the honey streaks to absorb moisture, and this will provide *O. agrili* adults with sufficient water. No more than 5-6 streaks are needed per enclosure for a 1-wk period.

Healthy EAB eggs, deposited on filter papers (Fig. 5), can be provided to *O. agrili* females beginning on the day of their emergence. Groups of up to 20 females can be held together in a single 16 oz. plastic cup, stocked with fresh EAB eggs once per week at a rate of 3-5 EAB eggs per *O. agrili* female. This generally results in parasitism rates of greater than 75%. Lower rates commonly result from using older EAB eggs (>3-4 days post-harvest), many of which will develop to near hatching during the course of exposure to *O. agrili* adults and will not be parasitized (Fig. 8). Groups of *O. agrili* adults in which no significant mortality has occurred, can be re-used for an additional week by moving them to a new,
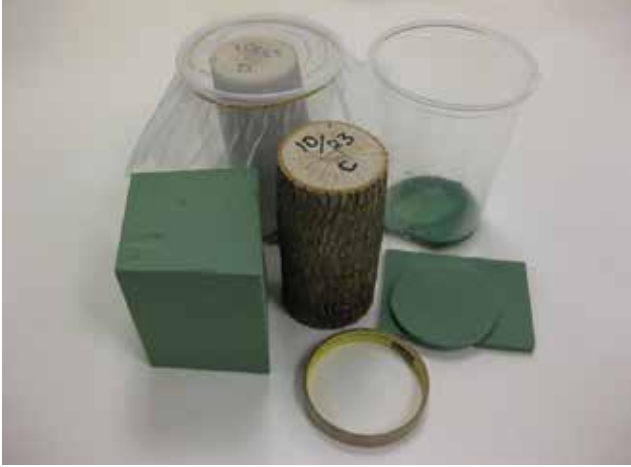
Figure 9. Supplies needed for creation of larval-bolt exposures for rearing emerald ash borer larval parasitoids. Clockwise from upper left: completed exposure cage with honey, empty cup with floral foam disk, sliced rectangle and circle cut with masking tape roll, floral foam brick and EAB-infested ash log (center). (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

Figure 10. Groups of *Spathius agrili* females are more efficient at parasitism and progeny production than single individuals. Several females often aggregate over unparasitized hosts; after parasitism occurs by one or more (often several) individuals, the group disperses and reforms over the gallery of another host. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

clean cup freshly streaked with honey, to which the appropriate number of EAB eggs have been added. Transferring *O. agrili* adults between cups is best accomplished by removing all other material from the cup (filter papers, dead wasps, and hatched EAB larvae), and then simply tapping the live wasps into the new cup. Fecundity decreases rapidly in groups of females more than two weeks of age and re-using adults for a third exposure is not recommended.

Spathius agrili

Spathius agrili Yang (Hymenoptera: Braconidae) is a gregarious idiobiont larval ectoparasitoid of EAB. Adult females are robust, capable of living for several months, and able to produce several dozen progeny during this time (Gould et al., 2011). At 25-27° C, one month is required from adult emergence to the production of new adults. New adult females need to be separated into groups of not more than 20-25 soon after emergence to prevent mortality from crowding. *Spathius agrili* can be easily housed in the same 473 mL cups as *O. agrili*, with the modification of using insect netting (www.skeeta.com, 625 holes per sq. in.) secured over the opening of the cup with a rubber band, rather than a solid lid. Honey is streaked into this screen (and replaced as it is consumed) and wasps are misted daily with clean, reverse osmosis water.

To mass rear *S. agrili* (and *Tetrastichus planipennisi* Yang as well, see below), EAB larvae are reared in small-diameter ash bolts in the laboratory until they reach the appropriate stage, and then host-infested logs are exposed to groups of adult wasps. Using a simple set of supplies (Fig. 9), EAB-infested ash logs can be exposed to parasitoids. The same 946 mL cups used to hold adult EAB beetles can be used here too, with minor modifications. Instead of a pad of paper towels, a disk of floral foam is added to the bottom of the cup to retain moisture. Floral foam bricks (http://www.save-on-crafts.com/artesia.html) are sliced into thin (4-6 mm) rectangular sections using a sharp knife. The inner cardboard circle from a roll of masking tape can be used as a circular "knife" to cut disks of floral foam from these rectangular slices; disks are then placed in the bottom of the plastic cups. The EAB-infested ash log is then firmly pushed into the floral foam, and clean water added until the foam is saturated. Wasps are added using an electric aspirator, a piece of insect netting is secured over the opening of the cup with a rubber band, and the netting is streaked with honey to provide nutrition to the adult wasps (Fig. 9). The setup is then held at 25-27° C and 75-80% relative humidity, under a 16:8 light-dark cycle, for one week. After this incubation period, the adult wasps can be removed with an aspirator and re-used in another exposure. The parasitoid-exposed log is then incubated under

the same conditions, and adults emerge in 2-3 weeks.

Using the methods outlined above (see section on EAB) to produce ash bolts bearing EAB larvae, logs set up to rear EAB should be exposed to parasitoids between days 24 and 26 post-setup, to ensure that the larval hosts have achieved the greatest possible mass, and yet have not burrowed into the xylem, where they are inaccessible to the parasitoids. Each rearing cage should be stocked with 10 *S. agrili* females and 2-3 males and an EAB-infested ash log. This group of wasps can be used for up to three additional 1-wk exposures to fresh EAB-infested ash logs. Groups of females often form oviposition aggregations and a group size of 8-10 is best to facilitate rapid parasitism of most hosts in a container (Fig. 10). Groups with moderate mortality (2-3 dead females) during their second and third exposures to hosts can generally be re-used, combining wasps as necessary to keep group size in the optimal range, provided no signs of disease, such as sporulating cadavers, are present. If any evidence of disease is detected, all wasps in the affected group should be discarded.

To store *S. agrili* during the winter, diapause can be induced during the wasp's larval stage by manipulating temperature and photoperiod (Belill and Lelito, 2011). However, emergence from diapause occurs over several months and is relatively unpredictable. Holding some wasps in diapause (at immature stages) is, therefore, not particularly useful for mass-rearing since production cannot be well enough timed to produce synchronized groups of parasitoids for release. The method does have some value for storing field-collected material for long periods and for limiting the number of generations that a colony is subjected to laboratory rearing. Adults from cocoons (containing mature larvae) stored under cold conditions for several months have lower fecundity and higher mortality (Gould et al., 2011). Methods for storing *S. agrili* that bypass the need for diapause are still under development.

Spathius galinae

Spathius galinae Belokobylskij & Strazanac (Hymenoptera: Braconidae) is a recently described EAB parasitoid from the Russian Far East and South Korea (Belokobylskij et al., 2012). It was imported to
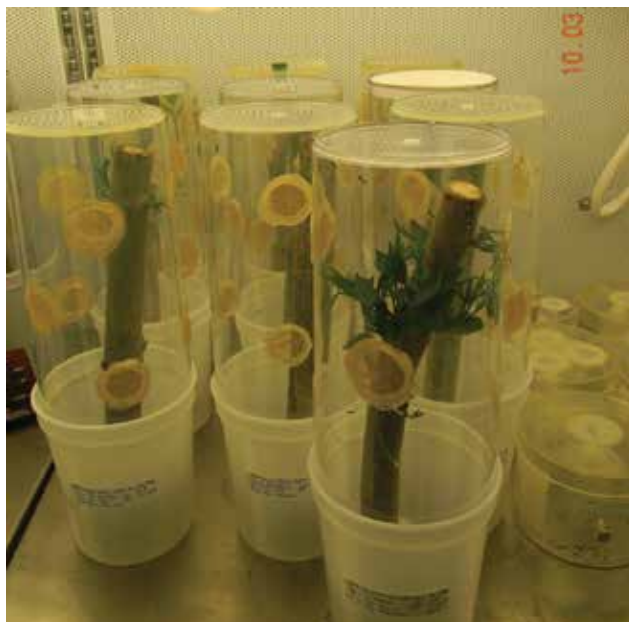
Figure 11. Exposure arena for production of *Spathius galinae*, containing five gravid female parasitoids and five males, as well as tropical ash logs infested with late instars of emerald ash borer. (Photo by Timothy Watt and Jian Duan (USDA ARS BIIR))

the USDA ARS Beneficial Insects Quarantine Facility (Newark, Delaware) in 2010 and its host range has been studied to estimate the safety of its release in the United States against the emerald ash borer (Gould and Duan, 2013). A petition for field release of *S. galinae* was submitted to the USDA APHIS and NAPPO in March of 2013 for regulatory review and approval. While not yet (January, 2015) approved for field-release, a positive response from NAPPO and USDA APHIS has been issued to the petition's scientists and the parasitoid's potential future mass-rearing is described here. Based on its distribution in the Russian Far East and other part of northeast Asia, climatic matching suggests that it is more suitable for introduction against emerald ash borers in the northeast United States and Canada (Duan et al., 2012; Gould and Duan, 2013) than the previously introduced Chinese parasitoids (e.g., Liu and Bauer, 2007; USDA APHIS, 2007).

Specific rearing methods have recently been developed at the USDA ARS Beneficial Insects Research Unit. The first step is the exposure of mated female wasps to 3rd-4th instars EAB larvae naturally reared on freshly cut green or tropical ash logs (Fig. 11). *Spathius galinae* takes about one month (29 d)

to complete a generation (from egg to adult) under laboratory conditions (25 ± 1° C, 65 ±10% RH, L:D 16:8 h photoperiod). During this period, *S. galinae* larvae molt four times to reach the 5th instar, which then spins a cocoon for pupation and development to the adult stage. Adult female wasps survive seven weeks on average, with the peak oviposition occurring after three weeks when wasps are reared in groups, or after two weeks when wasps are reared as single pairs. Throughout its lifespan, one *S. galinae* female produces an average of 31 progeny (range 12-41) when reared in groups, but many more offspring (ave. 47, range 5-94) when reared as single pairs. Thus, in mass rearing *S. galinae*, adult wasps can be exposed to hosts for several weeks. Although *S. galinae* can be reared in emerald ash borer larvae in either green or tropical ash sticks, the rate of non-emergence of *S. galinae* progeny was much higher (20%) when wasps were reared on hosts in green ash sticks than in tropical ash sticks (2.1%).

Temperatures below 15° C induce mature *S. galinae* larvae (inside cocoons) to enter an obligatory diapause. Once in diapause, a minimum of 1-3 months of chill at 3-12° C is required to break diapause and permit development to the adult stage. Specific mechanisms that induce and break diapause for this species are still being investigated. Unlike *S. agrili*, we have found that material can be stored up to six months with no decrease in adult emergence; however, it is not known if cold storage affects fitness or performance of adults.

Tetrastichus planipennisi

Tetrastichus planipennisi (Hymenoptera: Eulophidae) is a gregarious koinobiont larval endoparasitoid of EAB. The rearing method for *T. planipennisi* is similar to that for *S. agrili*, with a few key differences. First, rearing logs used should be from smaller trees whose bark is not more than 3 mm thick (Ulyshen et al., 2010; Abell et al., 2012). Second, the number of parasitoid adults per exposure cage should be slightly larger (12-15) than that used for *S. agrili*. Third, ash logs used to rear EAB larvae should be exposed to *T. planipennisi* a few days earlier (22-24 days post-setup of the EAB rearing) than for *S. agrili* to ensure

Figure 12. When nearly mature, the larvae of *Tetrastichus planipennisi* confer a braided appearance on their host larva. At this point or just afterwards, *T. planipennisi* broods can be induced into a state of torpor and be stored at low temperatures for up to six months without significant mortality. (Photo credit: Jonathan Lelito, USDA APHIS PPQ)

that the wasps have time to locate and parasitize all available EAB. Finally, groups of *T. planipennisi* females should only be used twice, as further use results in fewer progeny. Rearing of *T. planipennisi* is optimal at 25° C, 75-80% relative humidity, and a 16:8 light-dark cycle. Honey should be available to insects at all times, streaked on lids of rearing containers.

Another key difference between *T. planipennisi* and *S. agrili* is that *T. planipennisi* can be induced into a torpor state late in larval development, and in this condition *T. planipennisi* can be stored for long periods at 4 (±1° C) provided relative humidity is >75-80%. To induce torpor, immature parasitoids are chilled to 10° C on day 14 after host exposure to adult wasps, which is approximately when *T. planipennisi* larvae break out of their host larvae (Fig. 12). Logs can be held on moist floral foam in the rearing cages or they can be transferred into trays of shallow, clean water. Seven days after being placed at 10° C, rearing logs are transferred (remaining on moist foam or in shallow, clean water) to 4° C for storage. Mortality is generally <10% of the total cohort in each log when stored for up to six months. Under these conditions, most deaths are caused by bark drying and contracting, which crushes or traps some insects. Independent of bark desiccation, mortality increases during storage, as insects deplete metabolic reserves. Following return of stored wasps to 25° C,

T. planipennisi adults will usually emerge within 14 days, although some emergence may occur through day 21. Thus, unlike diapausing *S. agrili*, larvae of *T. planipennisi* can be stored in a manner that allows emergence of wasps to be predicted and controlled.

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CHAPTER 9: LIFE TABLE EVALUATION OF CHANGE IN EMERALD ASH BORER POPULATIONS DUE TO BIOLOGICAL CONTROL

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BACKGROUND

Emerald ash borer, *Agrilus planipennis* Fairmaire (EAB), is an invasive buprestid native to northeastern Asia that feeds on ash trees (*Fraxinus* spp.). First detected in North America (in Michigan, United States and Ontario, Canada) in 2002, EAB has spread rapidly, in part because of movement of infested nursery stock and untreated firewood (Cappaert et al., 2005a; BenDor et al., 2006; Poland and McCullough, 2006). As of January 2014, EAB was known in an additional 21 U.S. states and one Canadian province (USDA-APHIS, 2014), and it is expected to continue spreading to other parts of the continent with ash trees and a suitable climate (Sobek-Swant et al., 2012; Vermunt et al., 2012; DeSantis et al., 2013). Thus far, EAB has killed tens of millions of ash trees, with tree death generally occurring within 3-4 years of initial infestation by the beetle (Poland and McCullough, 2006). The treatment, or removal and replacement of landscape trees affected by this pest is projected to cost over $10 billion in the United States in the coming decade (Kovacs et al., 2010). While insecticide treatments can be effective at reducing losses from EAB in urban settings, biological control might represent the most sustainable option for suppressing populations at the landscape level and in natural environments over the long term.

The primary risk factor for North American ash is their limited innate host resistance to EAB (Liu et al., 2003; Bauer et al., 2005; Gould et al., 2005; Rebek et al., 2008; Herms and McCullough, 2014). Another cause of high ash mortality in North America is the lack of host-specific EAB natural enemies. In EAB's native range, however, parasitoids cause a considerable proportion of EAB egg and larval mortality, potentially regulating host population densities (Liu et al., 2003, 2007; Bauer and Liu, 2007; Wang et al., 2010; Duan et al., 2012a). In 2007, the USDA started the EAB Biological Control Program (Federal Register, 2007; Bauer et al., 2008), and began releasing three EAB parasitoids from China in the United States. These biological control agents are the larval ectoparasitoid *Spathius agrili* Yang (Yang et al., 2005, 2010), the larval endoparasitoid *Tetrastichus planipennisi* Yang (Liu et al., 2003, 2007; Yang et al. 2006; Duan et al., 2011a), and the egg parasitoid *Oobius agrili* Zhang and Huang (Zhang et al., 2005; Liu et al., 2007; Duan et al., 2011b; Duan et al., 2012b). Understanding the basic population dynamics of EAB will enable us to assess the effects of parasitism on EAB population growth and to more effectively target different life stages with biological control agents. One widely used approach to examining population dynamics is through life table analysis. Here, we briefly introduce some of the basic concepts of life tables, and then review some of the methods and results from life table analyses involving EAB.

LIFE TABLES

Life tables are constructed from data on the numbers of individuals that enter or die in different age or stage classes of populations over the course of a

generation (Van Driesche and Bellows, 1996; Stiling, 2012). Life tables have been used in ecology for over 65 years (Deevey, 1947), and they have provided insight into the population dynamics of a range of taxa including plants (Harcombe, 1987), fish (Cortes, 2002), mammals (Sherman and Morton, 1984), reptiles (Crouse et al., 1987), and insects (Hawkins et al., 1997). Early work in this field involved constructing life tables for the winter moth (*Operophtera brumata* L.) (Lepid.: Geometridae) (Varley and Gradwell, 1960; Buckner, 1969), and life tables have subsequently been employed widely in applied entomology for assessing the impact of pest control measures (Gould et al., 1992; Hoddle and Van Driesche, 1999; Kuhar et al., 2002; Nielsen et al., 2008). These effects can be examined either experimentally by manipulating certain sources of mortality in the field, or by modelling population dynamics with and without factors of interest in the model. For a more detailed discussion of life tables in general see Bellows et al. (1992) or Southwood and Henderson (2000).

Types of Life Tables

Two main types of life tables are utilized used in ecology: horizontal (cohort) and vertical (static) life tables (Van Driesche and Bellows, 1996; Stiling, 2012). Horizontal life tables follow a given cohort of same-aged individuals from birth throughout their lives, while vertical life tables use data from a population at one particular point in time. Vertical life tables often are used when study organisms are long-lived and it is not practical to follow them throughout their lives, and vertical life tables are

Table 1. Life table for an experimentally established EAB cohort at Legg and Central Parks, Meridian Township, Michigan in 2010.

Life stage	l_x	m_x	d_x	d_i	Mortality factor	q_x	q_i	q
(egg)	229	0	69		Infertility/predation/parasitism by *Oobius agrili*	0.300	0.300	0.300
L1-L2	160	0	34	34	Killed by tree resistance	0.213	0.557	0.149
L3	126	0	22	15	Killed by tree resistance	0.175	0.119	0.066
				3	Undetermined disease/other		0.024	0.013
				4	Parasitism (*Atanycolus* spp.)		0.068	0.018
L4	104	1	32	4	Undetermined disease/other	0.308	0.038	0.018
				7	Killed by tree resistance		0.067	0.031
				18	Parasitism (*Atanycolus* spp.)		0.173	0.079
				1	Parasitism (*Balcha indica*)		0.010	0.004
				2	Woodpecker predation		0.019	0.009
JL	71	9	52	52	Woodpecker predation	0.732	0.732	0.228
Adult exit hole observed	10	9	1	1	Undetermined disease/other	0.000	0.100	0.004
(Overwintered L4/JL-pupae)	10	-	3	3	29% additional overwintering woodpecker predation	0.290	0.290	0.013
(Emerging adults)	16	-	1	1	5% adult mortality from disease and predation	0.050	0.050	0.004
(Females)	8	-	-		1:1 sex ratio			
(F$_1$ eggs)	812	-	-		101.5 eggs per female			
R$_0$	3.6	-	-					

more suited for continuously breeding organisms with overlapping generations. Either method may be used for EAB, but given that we are able to create experimental cohorts of larvae, the horizontal (or stage-specific) life table may be more suitable.

Life Table Parameters

Several different parameters are used in life tables, and here we follow the general methods and column definitions described in Southwood and Henderson (2000). Table 1 is presented as an example of a stage-specific life table for EAB constructed from data collected in Michigan. Column headings are l_x = number of live EAB entering each stage (based on reverse calculation of the different stages of EAB observed at the sampling time, and with l_0 representing the number of eggs estimated to start the life table); m_x = number of live EAB observed at sampling time, d_x = number of dead EAB observed in each stage; q_x = apparent (stage-specific) mortality rate (d_x/l_x); d_i = number of EAB dying in association with the specific factor observed, q_i = apparent mortality rate because of the specific biotic factor di/lx); q = real mortality (d_x or d_i/l_0), R_0 = net reproductive rate, calculated as the ratio of l_0 divided by l_{F_1} (the number of eggs produced by surviving adults). R_0 can be interpreted as follows: if $R_0 = 1$, the population is constant; if $R_0 > 1$, the population is growing; and $R_0 < 1$, the population is declining.

Apparent Mortality

Expressing the number of individuals dying in a stage as a percentage of the number entering the stage generates the estimate of apparent mortality (Van Driesche and Bellows, 1996). Apparent mortality can subsequently be used to calculate k-values, as k = -log(1 – apparent mortality). Apparent mortality is generally used to estimate a single source of mortality within an individual life table, while k-values are additive over several mortality factors within a given life table and can be used to identify the key mortality factor for a population if life tables are available for a series of generations.

Marginal Attack Rates

For situations in which there are multiple contemporaneously acting mortality factors (e.g., predators consuming prey, some of which have already been parasitized), calculating the marginal attack rate is an improvement over apparent mortality (Elkinton et al., 1992). A marginal attack rate is the proportion of individuals entering a stage that are subject to attack by a given factor (A), even if some other factor (B) ends up actually killing some individuals previously attacked by factor A. It can be calculated using the following equation: $m_i = 1 – (1 – d)d_i/d$. This may be especially important with EAB because it is likely that some EAB larvae are stung by parasitoids but later consumed by woodpeckers or other insectivorous birds before immature parasitoids complete their development and kill their host.

CONSTRUCTING LIFE TABLES FOR EAB

EAB Life Cycle

Constructing life tables for EAB requires detailed knowledge of the species' life cycle (see Chapter 1). EAB females generally produce about 100 eggs (Wei et al., 2007; Wang et al., 2010), which are laid underneath small bark flakes or in crevices on ash trees. In Michigan, EAB's peak oviposition period occurs during late spring through summer (Poland and McCullough, 2006), and this appears to be the case throughout most of its North American range. Upon hatching, larvae burrow into the cambium and feed on the phloem and outer sapwood. Larvae develop through four instars in summer and fall, form a pupal chamber or cell (see Chapter 1), and overwinter in an obligatory diapause as mature 4th instar larvae. Under some circumstances, larvae require two years to complete development (Cappaert et al., 2005a,b) (see further discussion of this below). Adults begin emerging from ash trees in late spring or early summer (Brown-Rytlewski and Wilson, 2005) and feed on ash foliage throughout their lives. EAB adults mate within days of emerging, and oviposition typically begins after another week or two depending

on weather conditions (Cappaert et al., 2005a).

Given the cryptic nature of wood-boring insects, the life cycles of beetles such as EAB present many challenges for the construction of life tables. For instance, because EAB's eggs are laid between layers of bark and in bark crevices, they are not easily counted by observers. Furthermore, the majority of the EAB life cycle takes place as larvae feed, develop, and pupate inside host trees. This effectively prohibits repeated sampling as larval fates can only be determined by debarking trees, making it impossible to determine exactly when individuals might have died (although using stage-specific life tables can circumvent this). Additionally, wild EAB populations can be either univoltine (one-year generation time) or semivoltine (multi-year generation time), which appears to be influenced by climate, host tree condition, and oviposition date (Cappaert et al., 2005a,b). Having populations with mixed generations presents problems because individuals may not be exposed to a specific mortality factor for the same length of time or during the same season. For example, semivoltine larvae developing over two growing seasons will have a longer period of exposure to parasitoids than univoltine larvae that complete develop after one growing season.

Life table analyses for EAB have been conducted in Maryland, Michigan, and New York. In Michigan, a life table approach was used to assess the effectiveness of biological control agents released over three generations of EAB (Duan et al., 2010; Duan et al., 2014). In Maryland and New York, a life table approach was used by Jennings et al. (2013), primarily to explore the effect of woodpecker predation on EAB populations, although parasitism rates from biological control agents were also quantified.
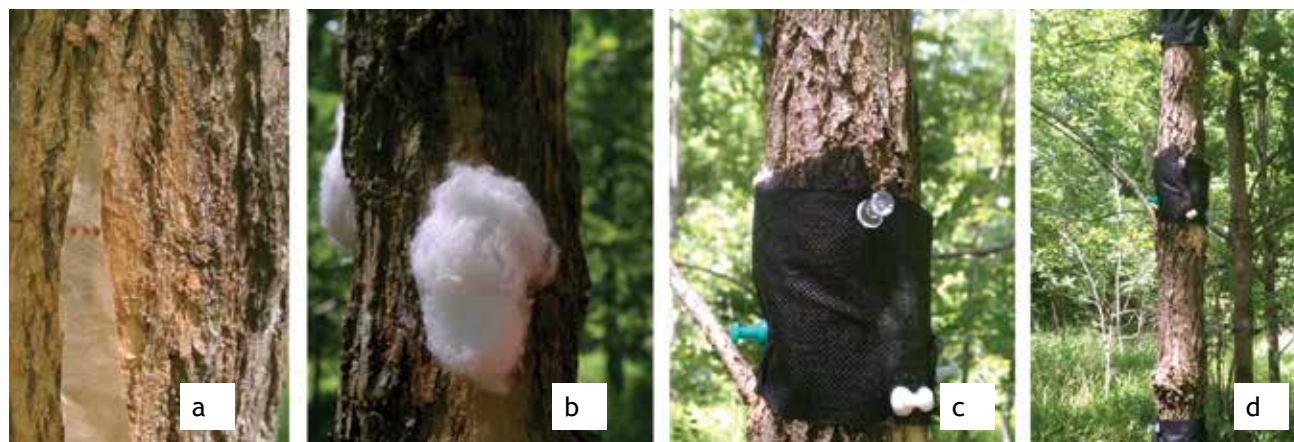
Establishing Experimental Cohorts

Several methods have been created to establish experimental cohorts of EAB in ash trees, which subsequently enabled accurate quantification of their population dynamics. For example, Duan et al. (2010) used two methods to establish EAB cohorts in Michigan. Their first method involved the placement of laboratory-reared EAB eggs directly onto the tree. To achieve this, EAB adults were first induced to

lay eggs underneath strips of ribbon on small ash logs in the laboratory (the ribbon simulating loose bark crevices found naturally on ash trees). Using a utility knife, small bark flakes (to which at least one egg was attached) were then cut from the logs and taken to the field. Bark flakes were inserted under bark flaps cut into ash trunks with knives, and the flaps were then pinned to the tree to offer protection from predators but still allow enough space so as not to crush the eggs. This method is labor intensive as it requires the production of eggs in the laboratory, and resulted in rates of EAB larval establishment of 14-26% (Duan et al., 2010). On the positive side, this method may retain some of the contact pheromones from EAB females, and it allows for placement of precise numbers of eggs in the field.

The second method utilized by Duan et al. (2010) was to cage gravid EAB females on trees (along with males and ash leaves), which forced oviposition to occur within a specific region of the tree. Cages were constructed from ventilated, rectangular containers (10 cm long x 7 cm wide x 4 cm deep) that were fastened to the trees, with the open side facing the trunk. One female and one male were placed into each cage. Benefits of this method were that it again allows for the retention of any contact pheromones from adult beetles, and it allows EAB females to oviposit naturally onto the bark. This method resulted in a higher rate of establishment in comparison with insertion of eggs into bark flaps, with ~75% of eggs producing established larvae (Duan et al., 2010). This method can be problematic, however, because quantifying the exact number of eggs produced is challenging, as some eggs may be overlooked or damaged during sampling via debarking. Furthermore, because of the variation in the number of eggs produced by females and consequently in larval density, statistical comparisons among trees can be difficult.

A third method, used by Jennings et al. (2013), modified the approach from Duan et al. (2010) that involved grafting individual EAB eggs directly onto the tree (Abell et al., 2012). For this method, eggs were first laid on a coffee filter paper substrate by EAB females in the laboratory. Strips of filter paper containing 1-3 fertilized eggs (as indicated by

Figure 1. Method for establishing experimental cohorts of EAB on ash trees. Shown are: (a) attaching eggs to bark, (b) covering eggs with cotton balls, (c) protecting eggs further with tree wrap, and (d) repeating on tree as desired, ensuring that bands of eggs are evenly distributed to avoid overlapping galleries (Photo credit: David Jennings, University of Maryland)

brownish color) were then cut and transported to field sites. Once suitable trees were identified at field sites, small patches of bark were shaved flat using a draw knife. Egg strips were then glued flush to the bark using standard wood glue, taking care to ensure that no glue came into contact with the eggs (Fig. 1a). To reduce the chances of galleries overlapping, a maximum of three eggs were placed on any one bark patch. Once the egg strip had been attached, a cotton ball was glued over the eggs, to reduce the risk of predation (Fig. 1b). This was replicated until there were six eggs at a particular height on the tree (a "band"). Once a band was completely inoculated with eggs, it was covered in tree wrap to limit predation (Fig. 1c). This process was repeated until there were five bands, each containing six eggs, on the tree for a total of 30 eggs (Fig. 1d). This method allows for a more precise number of eggs to be deposited on each experimental tree section. However, it appears to lower the rate of eggs transferring into established larvae, with estimates of establishment being ~54% (Jennings et al., 2013). Producing the eggs for this method is also labor intensive as it again requires the production of eggs in the laboratory, and care is needed when cutting the filter paper into strips. Additionally, this method precludes predation on the eggs themselves, removing this mortality factor from the life table. However, anecdotal evidence suggests that use of uncovered egg strips results in extremely high egg mortality, likely from predation.

Wild Populations

Life tables can also be constructed by directly sampling survival of life stages in wild populations of EAB (Jennings et al., 2013; Duan et al., 2014) by debarking sections of the tree and following the same process for identifying sources of mortality as with the experimental populations described above. However, several key caveats must be acknowledged when using wild populations for life table construction. The most obvious is that it is not possible to be certain how many eggs were laid on a tree in that year, given the difficulty associated with locating every egg on a tree and dating them. One approach to provide an estimate of the number of eggs per tree could be to search a given area of a tree and then extrapolate those findings for the rest of the tree. Additionally, it is not possible to state with certainty whether populations being studied are univoltine or semivoltine. The latter is strongly suggested if debarking of trees in the fall reveals many early instar larvae (likely being young of the sample year, whereas older larvae would have originated in the previous year and hence belong to a different generation). Despite these problems, constructing life tables for EAB in heavily infested areas can still provide valuable information on population dynamics. At such areas, it might not be possible to determine the fate of experimental cohorts given the high density of other galleries, and monitoring wild populations may be the most effective option at present.

Assigning Individuals to EAB Life Stages

Eggs. EAB eggs are around 1 mm in diameter and change color from white to brown a few days after being laid (Bauer et al., 2004; Wang et al., 2010). Such eggs found between layers of ash bark are presumed to be those of EAB.

Larvae and pupae. EAB larvae create characteristic serpentine galleries (Bauer et al., 2004; Lyons et al., 2004). Because the gallery size of EAB larvae changes over time, it can be used to estimate the stage the larva was in when it died, for example <2 mm wide for 1st to 2nd instars, 2-3 mm wide for 3rd instars, and >3-4 mm wide for 4th instars. Larvae then chew a pupation chamber in the outer sapwood or bark before folding into a J-shape for overwintering. These mature 4th instar larvae are termed J-larvae (Duan et al., 2010), but are sometimes referred to as prepupae (Chamorro et al., 2012) (see Chapter 1 for clarification).

Adults. For the purposes of life tables, EAB can be assigned as adults if there is evidence that they have successfully emerged from their pupal chamber, as indicated by a D-shaped exit hole in the bark. Adults are generally <10-13 mm in size and bright metallic green in color, and can live for 3-6 weeks after emergence (Cappaert et al., 2005a; Parsons, 2008).

Estimating Fecundity

Estimating fecundity from experimentally established cohorts or wild populations is extremely difficult, and thus far EAB life table studies have used fecundity data collected from laboratory-reared females. However, one problem with using estimates from laboratory-reared females is the high variation in the number of eggs produced. While some estimates have suggested that EAB females produce about 100 eggs (Wei et al., 2007; Wang et al., 2010), other data from laboratory-reared EAB showed an average of 74 eggs (range of 1 to 307 eggs per female (EPPO, 2013). Given the optimal rearing conditions in laboratories, it is possible that these are overestimates compared to field conditions. Alternatively, when logistically possible, sections of trees where EAB cohorts have been placed could be caged to trap and collect emerging adults. These adults could then be reared in the laboratory to obtain direct estimates of fecundity. However, cages would need to be checked frequently to minimize adult mortality.

Assigning Deaths in EAB Life Stages to Particular Mortality Factors

Eggs. Several methods have been developed to assess egg mortality under field conditions. One simple method involves searching for EAB eggs between thin layers of bark, typically for a set period of time, which then avoids problems associated with tree size (Duan et al., 2011b; Bauer et al., 2012; Duan et al., 2012b). Alternatively, estimates of egg mortality can be obtained through the use of egg sentinel logs (ESL). ESLs can be created in the laboratory using small logs with EAB eggs either laid directly onto the log surface by females or, if eggs have been laid on filter paper, attached artificially to the log. ESLs can then be suspended from trees in the field. Eggs exposed in this manner, however, often suffer high levels of predation unless protected with screening or ribbon.

Another method for assessing rates of egg parasitism in the field is carried out by scraping off outer sections of bark from ash trees and returning bark removed from delineated areas of the trunk to the laboratory (Bauer et al., 2012). There, samples are first placed in incubators for several weeks to allow live parasitoids time to emerge. Next, the bark scrapings are passed through standard window screening (~1 mm x 1 mm mesh) and the material passing through the screen is examined under a microscope to detect eggs and determine their fate (live, dead, dead parasitoid, emerged parasitoid, emerged EAB larvae, infertile egg). Eggs that have been parasitized often turn darker in color and contain droplets of meconium inside the egg shell, and parasitoids leave characteristic round exit holes. These two sources of information (emerged parasitoids and eggs found in screened material) are combined to estimate of parasitism. This procedure provides the best available estimate of parasitism, but it does not capture any estimate of predation rates on eggs. Predation (potentially by taxa such as ants or thrips) is indicated by large, jagged holes in the

eggs, and can potentially obscure previous parasitism (Duan et al. 2011b). Separate sampling methods are needed to estimate the extent of egg predation under field conditions.

Larvae and pupae. Larvae and pupae are relatively easy to locate in comparison to the other EAB life stages. To begin with, when using experimentally established cohorts created from eggs laid on filter paper, hatching success can be ascertained by inspecting the paper for signs that the larvae chewed through it. If larvae do successfully emerge from eggs, four general sources of mortality can then be assigned to EAB larvae and pupae: 1) disease, 2) killed by tree resistance, 3) parasitism, and 4) predation (Fig. 2). Disease can be assigned by examining the cadaver for signs and symptoms of entomopathogenic fungi or other disease-causing pathogens (Liu and Bauer, 2006) (Fig. 2a). However, because diagnostic tests for pathogens are not done, however, this category also includes EAB killed by starvation or cannibalism. Tree resistance, which typically affects early (1st and 2nd) larval instars, can usually be identified by callous formation around the larval gallery (Fig. 2b).

There are several approaches used to detect parasitism, which is most often identified in late (3rd and 4th) larval instars and pupae. These methods include examining galleries for meconium left by parasitoid larvae, or finding parasitoid larvae, pupae, adults, or parasitoid pupal exuviae in galleries (Fig. 2c). In addition to detecting introduced parasitoids, the same approach also detects several native parasitoids that attack EAB in North America, and those parasitoids should also be considered in life table analyses, including *Atanycolus* spp., *Balcha indica* Mani and Kaul, *Spathius floridanus* Ashmead, and *Phasgonophora sulcata* Westw. (Bauer et al., 2005; Duan et al., 2009; Duan et al., 2012c; Duan et al., 2013a). Even if there are no obvious signs of parasitism, any live larvae or pupae collected when debarking trees should be found and maintained in incubators for adult emergence and identification. Live larvae damaged during sampling should be immediately dissected to detect possible immature parasitoids. This may also be preferable even for the live undamaged larvae because many such larvae

Figure 2. Examples of the four main mortality factors affecting EAB. Shown are: (a) disease/intraspecific competition; (b) killed by tree resistance; (c) parasitism, and (d) predation. (Photo credit: Jian Duan, USDA-ARS, and David Jennings, University of Maryland)

die of fungal diseases during the prolonged rearing period required for them to complete their life cycle (Bauer et al., 2012).

Predation from insectivorous birds such as woodpeckers generally occurs on late (3rd and 4th) larval instars and pupae (Cappaert et al., 2005c; Lindell et al., 2008; Jennings et al., 2013; Koenig et al., 2013). Woodpecker damage can be identified on the outside of the bark before peeling, and then galleries can be traced underneath to the point of attack (Fig. 2d). Because parasitism and predation both occur on late larval instars, there is a possibility that some evidence of parasitism could be lost through predation. Presently there does not appear to be any evidence that woodpeckers preferentially feed on parasitized or unparasitized larvae, but the number of parasitized larvae has been was found to be significantly higher when woodpeckers were excluded from trees with experimentally established
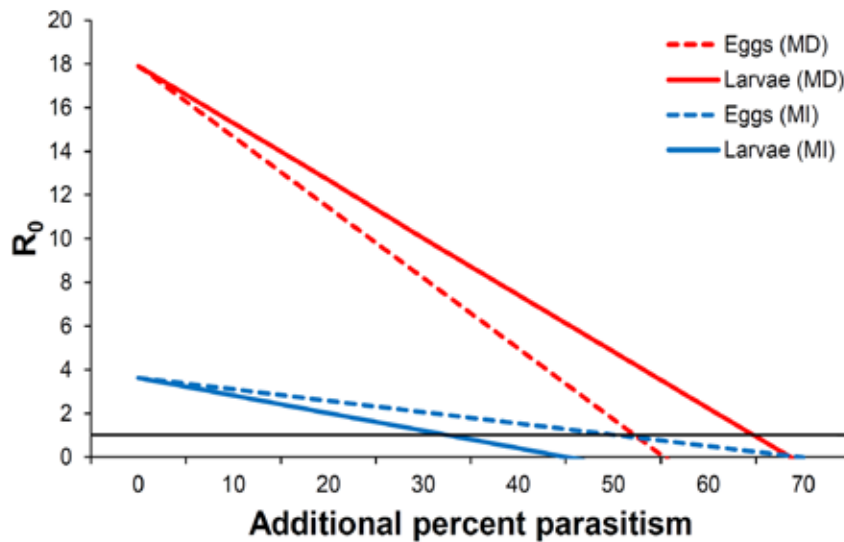
Figure 3. Simulated additional parasitism rates required to reduce EAB R_0 to <1 in Maryland (red lines) and Michigan (blue lines) (based on experimental cohorts at Legg and Harris Nature Center Parks in 2010). Solid lines represent larval parasitism, dashed lines represent egg parasitism. Black line represents R_0 =1, beneath which EAB population growth would be declining.

cohorts of EAB (Jennings et al., 2013) , suggesting that parasitized larvae are taken.

Adults. Estimating the mortality of EAB adults can be challenging. Natural sources of mortality likely include predation from insectivorous birds and disease, but these are difficult to quantify under field conditions. For the purposes of life table construction, the most effective way may be to use data collected from laboratory studies to parameterize the models. Such an approach was utilized by Duan et al. (2014), who assigned a mortality rate of 5% to EAB adults.

EFFECTS OF BIOLOGICAL CONTROL ON EAB POPULATIONS

In areas where EAB parasitoids have been released for several years there is evidence that some species (particularly *T. planipennisi*) are establishing and increasing in population size (Duan et al., 2013b). With few published studies examining the effects of these parasitoids on EAB population growth using a life table approach, it is difficult to make generalizations from the results. However, using the data available, we can manipulate life tables and

investigate how EAB populations are projected to change under different scenarios. Specifically, we can use the data from published life tables to model what rates of egg and larval parasitism would be sufficient to reduce EAB population growth to non-pest levels.

In Maryland, EAB first arrived in 2003 from EAB-infected ash nursery stock shipped from Michigan and sold in Maryland and Virginia. Despite an attempt to eradicate EAB in this region, EAB was considered established in Maryland in 2006 and Virginia in 2008 (see Chapter 1). In Maryland, EAB populations from experimental cohorts were found to have R_0 values of 17.9 when woodpeckers were present and 19.2 when woodpeckers were excluded using caging (Jennings et al., 2013). Both of these growth rates are high, and they suggest that woodpecker predation does not contribute greatly to mortality at sites with a low to moderate EAB infestation. The main source of mortality at these newly colonized sites was tree resistance, and while parasitism was detected it was at relatively low levels. However, in New York (at study sites where EAB was established longer in comparison to those used in Maryland), where neither parasitism nor tree resistance were significant sources of mortality, R_0

values for wild populations were much higher (29.8 and 50.5 when woodpeckers were able to feed on EAB stages and when woodpeckers were excluded, respectively) (Jennings et al., 2013). This suggests woodpecker predation can significantly reduce EAB population growth only at sites where EAB densities are high.

In Michigan (at sites where EAB has been established for several years), life tables constructed by Duan et al. (2014) found that in the first generation of EAB studied R_0 values were similar to those in Maryland (16 for experimental cohorts and 19.4 for wild populations). However, a large drop in R_0 was seen in the second generation (4.6 and 4.7 for experimental and wild cohorts, respectively), which coincided with an increase in the level of parasitism detected. Mortality was greater in later larval stages than in early ones, primarily because of parasitism from *Atanycolus* spp. and *T. planipennisi*. Host tree resistance and disease remained important for early larval stages, while woodpecker predation was the largest mortality factor for J-larvae. The results also suggest that experimental and wild cohorts of EAB may be used comparably for population studies if certain adjustments are made to account for potentially overlapping generations.

Models using data from experimental cohorts at some of the sites in Maryland and Michigan suggest that if egg and larval parasitism can be increased then there is the potential to reduce EAB population growth to more manageable levels (Fig. 3). These models were constructed by increasing parasitism in increments of 10%, while re-adjusting the stage-specific mortality rate to keep it at the originally observed proportions. Results suggest that in Michigan, ~30% additional larval parasitism would be sufficient to cause a decline in EAB populations, while in Maryland it would take ~65%. An increase in egg parasitism of ~50% would be sufficient to reduce $R_0 < 1$ in both locations. While these models are simplistic, they serve to illustrate the utility of life table analyses.

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CHAPTER 10: RISK TO ASH FROM EMERALD ASH BORER: CAN BIOLOGICAL CONTROL PREVENT THE LOSS OF ASH STANDS?

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INTRODUCTION

Ash trees (*Fraxinus* spp.) are an important components of both natural forests and urban plantings in the United States and Canada (Federal Register, 2003; Nowak et al., 2003). There are approximately16 species of *Fraxinus* native to North America (Harlow et al., 1996; USGS, 2014), each adapted to different ecological niches across a range of climates zones, soil types, and moisture gradients (Eyre, 1980). This abundant and diverse ash resource provides economic benefits, with ash timber alone valued at $282 billion (Nowak et al., 2003). For instance, green ash (*F. pennsylvanica* Marsh.), the most widely distributed ash in North America, is a fast growing, moderately shade tolerant tree that grows in mixed hardwood stands along river bottoms and wetlands, in small lowland groves, or in upland mesic sites. It was planted extensively throughout North America as an ornamental landscape and street tree due to its rapid growth and hardiness, and as agricultural shelterbelts for livestock shelter and soil conservation (MacFarlane and Meyer, 2005; D'Orangeville et al., 2008). Ash trees are also a valuable ecological component of the deciduous forests of eastern North America, and provide food, cover, nesting sites, and habitat for mammals, birds, insects, and other organisms (Poland and McCullough, 2006; Gandhi and Herms, 2010; Koenig et al., 2013).

Clearly, the ecological and economic value of ash in North America justifies appropriate measures for its protection against the invasive emerald ash borer (EAB, *Agrilus planipennis* Fairmaire), which threatens the persistence of ash in mixed hardwood stands. In this chapter, we will first review various ecological factors that may affect the potential for ash mortality due to EAB. We will then examine the population dynamics of EAB in its newly invaded region (North America) vs. its native range (northeast Asia), and attempt to identify critical or key biotic factors that may be employed or manipulated to suppress EAB population growth. Finally, we will review the current EAB biological control program that involves introduction and establishment of hymenopteran parasitoids from northeast Asia. In particular, we will examine whether natural enemies (parasitoids) can maintain EAB populations at an equilibrium density low enough to allow ash to regenerate and recover.

FACTORS AFFECTING ASH RISK FROM EAB INVASION

Ash trees were once relatively free of serious, major diseases (except for ash yellows in some limited areas) and insect pests in North America until the arrival of EAB (Barnes and Wagner, 2003; Pugh et al., 2011). EAB was first detected in North America in Michigan in 2002, and as of February 2014, it had been detected in 22 U.S. states and two Canadian provinces, killing millions of ash trees (see reviews in Herms and McCullough, 2014;) (Fig. 1) (see reviews in Herms and McCullough, 2014). All ash species native to North America that have been encountered by EAB to date are susceptible to EAB, including the most

common species: green, white (*F. americana* L.), and black (*F. nigra* Marsh.) as well as the less common blue (*F. quadrangulata* Michx.)and pumpkin ash (*F. profunda* [Bush] Bush). Although there is increasing evidence that EAB will attack all species of *Fraxinus*, innate susceptibility of ash trees varies with a variety of ecological factors such as physiological condition, habitat type, and species. Below are some ecological factors that may affect the likelihood of ash risk from EAB invasions in North America.

Figure 1. Mortality of overstory green ash trees caused by emerald ash borer in 2003, Kensington Metro Park, Brighton, Michigan. (Photo credit: Leah Bauer)

Ecological Habitats: Natural Forest vs.Urban Plantings

After its accidental introduction into North America, EAB established on ash trees in urban areas and subsequently spread into nearby natural forests (Haack et al., 2002; Michigan State University, 2014; Canadian Food Inspection Agency, 2014). Although EAB is a strong flier, long-range dispersal occurs primarily through human activities, often along roadways lined with ash trees. EAB spread has appeared to follow a wave pattern across the landscape through short-distance natural dispersal and as well as long-range dispersal assisted by human activities (Taylor et al., 2010; Prasad et al., 2010; Kashian and Witter, 2011). In Russia (Duan et al., 2012a; Straw et al., 2013) and northeastern China (Liu et al., 2007; Wei et al., 2004, 2007; Wang et al., 2010), EAB outbreaks have been noted primarily on North American ash trees planted in plantations or as street

trees. EAB populations have the potential to disperse quickly in urban areas due to widespread planting of susceptible ash species and human-assisted movement and storage of EAB-infested materials (see review in Herms and McCullough, 2014).

Age of Ash: Mature Trees vs Saplings

Although the diameter at breast height (DBH) of ash trees does not significantly influence the probability of EAB oviposition or infestation (Marshall et al., 2011; Klooster et al., 2014; Jennings et al., 2014), EAB infestations in North America have first killed mature (canopy) ash trees rather than smaller understory saplings (Cappeart et al., 2005). Recent studies have further shown that ash saplings with DBH <2.5 cm are rarely attacked by EAB (Marshall et al., 2011, 2013). It is conceivable that young ash saplings have both physical (e.g., smooth-bark surface) and chemical (secondary compound) characteristics that are less attractive to EAB oviposition than canopy ash trees (e.g., Marshall et al., 2013). It is also possible that saplings with stem diameters smaller than <2.5 cm are too small to be colonized and killed. Klooster et al. (2014) found that mortality of green, white, and black ash trees in mixed stands with stems equal to or greater than 2.5 cm exceeded 99% in southeastern Michigan forests by 2009, suggesting that there is little resistance or tolerance in these ash populations, and that EAB does not discriminate based on chemical or physical attributes when populations are high.

Species and Variety

Liu et al. (2003), studying EAB in China, reported higher EAB densities in North American species (green ash and velvet ash, *F. velutina* Torr.) than in Asian species (*F. chinensis* Roxb.; *F. chinensis* var. *rhynchophylla*). In a common garden trial in Michigan, Rebek et al. (2008) confirmed the presence of interspecific variation in responses to EAB infestations between the Asian (*F. mandschurica* Rupr.) and North American species (*F. pennsylvanica* and *F. americana*). Exposed to similar EAB infestation pressure, the Asian species, Manchurian ash, suffered far less mortality and yielded far fewer

Figure 2. Inter-species variation in ash resistance to emerald ash borer infestation in its native range (Vladivostok, Russia). North American green ash trees (*F. pennsylvanica*) planted in the 1970s on the left side of the tramline show late stages of EAB infestation symptoms (canopy declines, exit holes, bark splits etc.). Oriental ash (*F. rhynchophylla* or *F. manschurica*) were planted in the 1980s on the opposite side of the tramline show little signs of EAB infestation. (Photo credit: Jian Duan)

adult beetles than several cultivars of North American green and white ash. Duan et al. (2012a) also observed similar interspecific variation in resistance to EAB infestations between the Asian species *Fraxinus rhynchophylla* Hance and North American green ash (*F. pennsylvanica*) in the Russian Far East, the possible native range of EAB (Fig. 2). The higher resistance of Asian ash may have resulted from a long co-evolutionary history with EAB (Liu et al., 2003; Rebek et al., 2008), thereby restraining EAB densities within its native range.

Seed Banks and Regeneration

Kashian and Witter (2011) examined the potential for ash canopy tree recovery in EAB-affected stands from 2007 to 2009, measuring regeneration at 45 sites in southeastern Michigan (USA) following stand decline from EAB infestation. White, green, or black ash regeneration was abundant at all sites, particularly of the smallest ash height classes, but new seedling density dropped significantly between 2007 and

2009. This dramatic decrease in new seedlings was interpreted to be the result of a depleted seed bank, because few or no nearby mature ash trees existed to provide seed. Recent sampling in small pure stands of green ash suggest that seed production during ash mast years (on both surviving mature ash and sprouts from killed trees) may be sufficient to maintain a significant – though greatly reduced – pool of ash regeneration that may allow ash to persist at low levels (D. M. Kashian, unpub.). It remains to be seen if ash regeneration will be high enough to repopulate sites with mature trees in Michigan where pre-EAB ash density was lower, especially because the future dynamics of EAB populations in the region are still uncertain.

In another study, Klooster et al. (2014) conducted extensive soil sampling in southeastern Michigan forests located within 45 km of the epicenter of the infestation and found no seeds after 2007, suggesting depletion of the seed bank. Once mortality of ash with stem diameters greater than 2.5 cm exceeded 99% in 2009, they observed no newly germinated seedlings (with cotyledons) either inside or outside their plots, which is also consistent with a depleted seed bank. They did observe that ash mortality decreased slightly in 2010 to about 97% as smaller saplings grew large enough to reach the 2.5 cm size class. However, EAB trapping revealed that a low-density EAB population continued to persist on this cohort of saplings. Based on these patterns, Klooster et al. (2014) concluded that the long-term fate of ash in these sites will depend on the establishment of a dynamic equilibrium between current ash regeneration, EAB, and its natural enemies.

Natural Enemies

Several species of larval and egg parasitoids have been discovered in the native range of EAB. Field studies in Asia show that these natural enemies cause up to ~70% parasitism of EAB larvae or eggs in EAB's native range (Liu et al., 2003, 2007; Duan et al., 2012a). It is very likely that these Asiatic natural enemies exert important top-down effects on EAB population dynamics and may potentially limit outbreaks of EAB in Asia to levels that do not

cause significant mortality to ash. However, natural enemies do not appear to have prevented EAB outbreaks on highly susceptible North American ash species that were planted in China (Liu, 1966; Wei et al., 2004). In contrast, parasitism by North American parasitoid species was minimal (<5%) when EAB was first detected in Michigan and is often low in other newly infested areas (e.g., Bauer et al., 2005; Duan et al., 2009, 2012b, 2013a). This lack of effective natural enemies in North America was the justification for introduction of Asian parasitoids into the United States for classical biocontrol of EAB. Whether or not the newly introduced EAB parasitoids will provide sufficient reduction of EAB populations to allow recovery or regeneration of ash needs continued study as part of the EAB biological control program.

EAB POPULATION DYNAMICS IN NEWLY INVADED AND NATIVE RANGES

The invasion wave of EAB in ash-dominated forests of a newly invaded region has been described as having three main stages: the cusp, crest, and core (Burr, 2012; Burr and McCullough, 2012). The cusp phase occurs at newly infested sites in the first few years as EAB populations slowly build, before their numbers rapidly increase and cause tree mortality in the crest phase. The core phase then occurs around 10 years after the initial infestation, by which time most ash trees have died and EAB populations have crashed. Burr (2012) characterized EAB population density and conditions of green ash overstory and regeneration from 2010 to 2011 in 24 forests sites in Michigan, which were at the three different stages of the EAB invasion wave. Recent studies suggest that host tree mortality (or depletion of host tree resources) is the major factor driving the invasive population of EAB to emigrate or disperse into new areas or forests (Mercader et al., 2009; Burr, 2012; Burr and McCullough, 2012). However, long-term studies of EAB population dynamics and its underlying regulation factors at different invasion stages are currently lacking in North America. Evidence gathered thus far in the native range of EAB has shown that EAB outbreaks in northeastern Asia are rare events in natural forests, and outbreaks occur

primarily in isolated plantations or urban plantings of mostly North American ash (*F. pennsylvanica*, *F. americana* or *F. velutina*) (Wei et al., 2004). While infestations within the native range of EAB can occasionally cause significant ash mortality in urban plantings or plantations, no important outbreaks (comparable to those in North American forests) have been recorded in canopy ash in native Asian forests (Liu et al., 2007; Williams et al., 2010; Duan et al., 2012a). Recent ecological studies of EAB population dynamics in in the Russian Far East and northeastern China suggest that natural enemies (larval and egg parasitoids) and host tree resistance are the two key factors that regulate EAB population dynamics in its native range (Liu et al., 2003, 2007; Duan et al. 2012a), and thus the lack of these two key mortality factors in North America may explain the severity of EAB damage there. Understanding the ecological mechanisms or key factors that regulate EAB population dynamics in both its native range and newly invaded areas will be critical for developing sustainable strategies for managing this invasive pest in North America.

CAN BIOLOGICAL CONTROL SIGNIFICANTLY DECREASE ASH MORTALITY?

The Current Status

Classical biological control was initiated shortly after EAB detection in the United States due to the failure of eradication efforts (see Chapter 4). This program has introduced and achieved establishment of three exotic parasitoids (two larval parasitoids and one egg parasitoid) sourced from part of the native range in northeastern China (see Chapter 5). Field surveys in Michigan, Maryland, and New York show that one of the released larval parasitoids, *Tetrastichus planipennisi* Yang, became widely established on EAB populations at both release and nearby control sites 3-4 years after release (Bauer et al., 2008, 2009, 2010; Gould et al., 2011; Duan et al., 2012b, 2013b; Jennings et al., 2013). Duan et al. (2013b) showed that parasitism of EAB larvae by *T. planipennisi* in central Michigan steadily increased from <1% in the first year (2008)

after field releases to ~21% (release plots) and 12% (control plots) four years later (by 2012). While the introduced egg parasitoid, *Oobius agrili* Zhang and Huang, appears to have also established primarily at release sites in central Michigan and Maryland following releases between 2008 – 2011, rates of EAB egg parasitism varied from <5% to as high as ~28% among different release sites and years after field releases (Duan et al., 2010, 2012b; L.S. Bauer, unpublished data) and appear to be increasing. In contrast, establishment of the braconid larval parasitoid *Spathius agrili* Yang is less certain, and observed levels of field parasitism by this species have been minimal (<0.1%).

Currently, levels of parasitism by these introduced parasitoids are still much lower than those observed in their native range (Liu et al., 2007; Duan et al., 2012a; Yang et al., 2010). This is most likely due to the limited number of the wasps released in the initial phases of the program (2008-2011) and the short period of time available for the released parasitoids' populations to increase. With release of larger numbers of these parasitoids in North America in the next few years, it is possible that these parasitoids will greatly increase in abundance and inflict levels of EAB larval and egg parasitism that are comparable to those observed in their native ranges. With the development of effective mass-rearing methods, it is also conceivable that these biological control agents, particularly the egg parasitoid *O. agrili*, could be released in large numbers as a means of effectively preventing EAB populations from reaching levels able to kill ash trees, at least on a local scale.

The Premise of EAB Biological Control

The premise of EAB biological control is that EAB outbreaks are rare in China and other parts of EAB's native range, in part because of the presence there of more effective natural enemies that suppress outbreaks before they occur. It is commonly noted that there are many isolated stands of healthy saplings of North American ash species (green and velvet, respectively) in urban areas of the Russian Far East and China. It is thus plausible that EAB parasitoids in this region might have protected these susceptible ash trees at two different phases. First, saplings of

susceptible ash species in Asia might be colonized initially at low levels of EAB because there are fewer beetles coming from resistant trees, which would not be the case in North America. Moreover, survivorship of F_1 immature EAB stages on these saplings might be reduced by a rapid increase of parasitoid populations due to shorter handling times for parasitoids to attack hosts on saplings. This could retard EAB buildup on susceptible ash trees in Asia. Second, the abundance of EAB parasitoids in the native range may in fact allow their populations to increase rapidly via numerical response to incipient infestations of EAB on susceptible ash species and thus directly protect the trees while beetles are at relatively low density.

Factors Affecting the Efficacy of EAB Biological Control

The question then arises whether these introduced parasitoids can successfully establish in North America and effectively reduce the invasive EAB population to a sufficiently low level to allow ash for regeneration and recovery of ash overstory trees in forests. See Figure 3, a hypothetical model of EAB population dynamics with successful EAB biological control.

The following ecological factors are most likely to influence the success of the current EAB biological control program in North America:

Climatic matching and adaptability of the introduced parasitoids in North America.
The adaptability of the introduced Asiatic parasitoids to the climatic and other ecological conditions (e.g., host's phenology) in North America would have profound impacts on their successful establishment and efficacy in controlling EAB populations. Climatic matching analysis showed that the climatic conditions in northeast China, where *T. planipennisi* and *O. agrili* originated, generally matches that of the midwestern and northeastern United States, where EAB has firmly established (Federal Register, 2007). Thus, it is not surprising that both species appear to have become well established in Michigan and other midwestern states shortly after their field releases. However, the establishment of the other Chinese larval parasitoid, *S agrili*, has been less certain in Michigan and other northern states in the United States. This is most
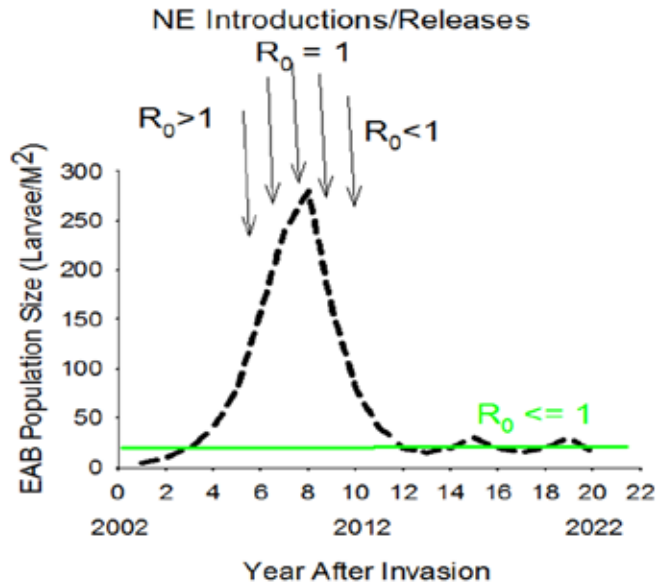
Figure 3. A schematic illustration of emerald ash borer population dynamics with successful biological control: Released parasitoids should reduce the EAB population to a low "equilibrium" density that allows ash regeneration and recovery to canopy trees.
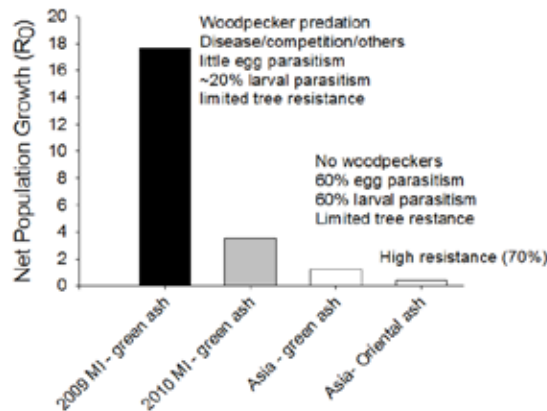
Figure 4. Comparison of net reproductive rate (R_0) of an emerald ash borer population in North America vs. Asia when different levels of dominant mortality factors are present or absent from life tables constructed in two study periods (2008–2009 and 2009–2010) in central Michigan, the epicenter of the North American invasion. $R_0 > 1$ results in population increase; $R_0 = 1$ results in a stable population; $R_0 < 1$ results in successful suppression of EAB population growth.

likely because the source of *S. agrili* was further south in Tianjin, China (southeast of Beijing on the coast) where temperatures are moderated by the China Sea. In contrast to *S. agrili*, the congener *Spathius galinae* Belokobylskij & Strazanac was collected from the Russian Far East and is thus likely to be more cold tolerant than *S. agrili* (Duan et al., 2012a; Belokobylskij et al., 2012; Khun et al., 2013). Climate matching analysis indicates that the climate in the central region of the United States is potentially more suitable for *S. agrili* than the more northern areas where most releases have been made to date. Climate matching analysis further indicates that the portion of the US suitable for *S. galinae* is considerably further north than for *S. agrili* (Gould and Duan, 2013).

The size or age of ash trees to be protected.
Ash trees are fast growing, an expected lifespan of 200 to 300 years, and normally produce seeds after 30 – 40 years (Garden Guide, 2014). Although the size or age of ash trees (often measured as DBH) does not appear to significantly influence the probability of EAB infestation or tree mortality (see previous section), bark thickness as a function of tree age or size can have significant effects on the efficacy of EAB larval parasitoids in finding and attacking host larvae. For example, Abell et al. (2012) showed that EAB larvae infesting ash trunks with a DBH>11.2 cm are rarely parasitized by the larval parasitoid *T. planipennisi* because this species has an ovipositor ranging in length from 2.0-2.5 mm and thus cannot oviposit through bark that is thicker than 3.2 mm. Based on these findings, Abell et al. (2012) recommend releasing *T. planipennisi* only in early-successional stands with small ash trees, but not in mature forests where ash was mostly larger. For protection of large ash trees (DBH >12 cm), parasitoids such as *S. galinae*, with much longer ovipositors (4.5 – 5.5 mm) should be considered for use in EAB biocontrol programs. Based on regression analysis of bark thickness and DBH (Abell et al., 2012), it is estimated that *S. galinae* could successfully oviposit through bark in trees up to 29 mm DBH, greatly enhancing its usefulness as a biological control agent.

Level of ash tree resistance to EAB.
Host tree resistance is a dominant factor that can have a "bottom-up" effect on EAB population growth in its native range (see previous section). Levels of host tree resistance will also have effects on the success of natural enemies, particularly larval parasitoids, in controlling EAB populations that have already infested ash trees. For example, there is strong evidence that EAB larvae develop more slowly and more often express semi-voltinism in healthy ash trees compared to artificially girdled, or previously-infested ash trees (McCullough et al., 2009; Duan et al., 2010). The slower larval development and a semi-voltine life cycle may provide a much wider of window for foraging parasitoids to attack host larvae, and thus result in higher control efficacy. A population dynamics model parameterized with observed larval and egg parasitism rates (~60%) in Asia, showed that natural enemies in Asia can quickly reduce the rate of EAB population growth when accompanied by moderate to high levelsof host plant resistance (Fig. 4) (JJD unpub; see also Chapter 9).

CONCLUSIONS

Ash trees were once relatively free of serious, major diseases and insect pests in North America until the arrival of EAB, which was first detected in North America in Michigan in 2002. As of February 2014, EAB had been detected in 22 U.S. states and two Canadian provinces, killing millions of ash trees. The ecological and economic value of ash justify appropriate measures to manage this invasive pest, and the current EAB biological control program was initiated shortly after its detection in the United States due to the failure of eradication efforts. The premise underlying the classical EAB biological control program is that EAB outbreaks are rare in China and other parts of its native range, in part because effective natural enemies prevent or quickly suppress EAB outbreaks.

The EAB biological control program has resulted in the introduction and successful establishment in North America of three exotic parasitoids (two larval parasitoids, *T. planipennisi* and *S. agrili,* and one egg parasitoid, *O. agrili*) sourced from the native range of EAB in northeastern China. An additional species of EAB parasitoid, *S. galinae*, is also currently under review for potential release against EAB in the

northeast United States. The key question is whether these introduced parasitoids, once established, can effectively regulate the EAB population at sufficiently low densities such that an equilibrium can be maintained between EAB and its natural enemies that permits survival and regeneration of ash in North American forests.

To answer this question, we examined various factors that may potentially affect the risk to ash from EAB invasion, including population dynamics of EAB in both the newly invaded region and its native range (northeast Asia), and dominant biotic factors that regulate EAB populations in its native range. We suggest that ash tree resistance and natural enemies (parasitoids) are the two dominant biotic factors that have the potential to regulate EAB population dynamics. A population dynamics model parameterized with the egg and larval parasitism rates (~ 60%) observed in EAB's native range suggests that natural enemies coupled with moderate to high levels of host plant resistance has the potential to reduce the growth rate of EAB populations below replacement, and thus maintain EAB populations at low-density, equilibrium levels, which should permit survival and regeneration of ash in the aftermath of EAB invasion in North America forests.

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CHAPTER 11: FUTURE DIRECTIONS IN EAB-AFFECTED FORESTS

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The ability of natural enemies to slow emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), population growth in a given area will play a major role in determining whether many native ash species can persist as functional components of forest ecosystems. Population growth of EAB, like that of any other organism, is determined by reproduction, development rate, and survival at each stage of development. Fecundity of female EAB is relatively high when compared to other phloem-feeding *Agrilus* species. For example, *A. difficilis* Gory can produce 36 eggs per female (Akers et al., 1986), *A. anxius* Gory, 55 eggs (Rutledge and Keena, 2012), *A. auriventris* Saunders, 140 eggs (Huangfu et al., 2007), while a female *A. auroguttatus* Schaeffer may produce 575 eggs (Lopez and Hoddle, 2014). In laboratory settings, EAB females can lay more than 275 eggs over the course of their life span. Although egg viability tends to diminish over time, even in the wild, on average, EAB can probably produce at least 40-60 offspring per female.

Most EAB larvae develop in a single year, but in newly infested ash that are relatively healthy a high proportion of larvae require two years to develop (Siegert et al., 2010; Tluczek et al., 2011), initially slowing the new population's growth rate (Mercader et al., 2011). Populations of EAB in northern latitudes where summers are short may also be more likely to require two years for development, a pattern previously observed with *A. anxius* (Barter, 1957). In stressed ash, including trees injured by increasing densities of EAB larvae, however, all or nearly all EAB develop in a single year (Tluczek et al,. 2011). Like other phloem-feeding insects, the survival of larvae of EAB is primarily limited by the availability of phloem of its host tree. Using data from several field studies, Mercader et al. (2011) estimated that an EAB larva requires approximately 10 cm^2 of ash phloem to complete development. Similarly, McCullough and Siegert (2007) reported an average of approximately 89-105 adult EAB could develop per m^2 of phloem in white ash (*Fraxinus americana* L.) or green ash (*Fraxinus pennsylvanica* Marshall). Canopy decline generally becomes apparent at a density of 25-35 EAB per m^2 (Anulewicz et al., 2007). Ash phloem available for larval feeding increases rapidly with the diameter at breast height (dbh) of the tree. For example, using methods of McCullough and Siegert (2007), a tree with a dbh of 30 cm can produce approximately 1335 adult EAB, while a 60 cm tree can produce 6285 beetles. Of course, not every m^2 of phloem will produce 90-100 adult EAB beetles. At the peak of the invasion, individual trees can harbor 200-300 early stage larvae per m^2 (Tluczek et al., 2011, Tanis and McCullough 2015), but intraspecific competition for phloem results in high mortality, typically of third instars. Nevertheless, when ash, particularly large ash, are abundant, EAB density in a given area will be very high during the peak of the invasion wave.

Given that few options are available for reducing female fecundity or slowing development of EAB, effective control tactics must limit survival of eggs, larvae, or adult beetles. Systemic insecticides protect landscape ash trees by substantially reducing survival of EAB adults and larvae, but these products are obviously not likely to be used in forests. Mortality of EAB attributable to parasitism and predation varies considerably among sites and among trees within sites. Relatively high rates of egg parasitism (ca 20%) (Abell et al., 2014), larval parasitism (10-70%) (Cappaert and McCullough, 2009; Duan et al., 2013; Tanis and McCullough, in press 2015), and

woodpecker predation (22-85%) (Lindell et al., 2008; Jennings et al., 2013; Duan et al., 2013; Flower at al., 2014; Tanis and McCullough, 2015) have been recorded at some sites in Michigan and Ohio. Duan et al. (2014) reported that in some Michigan sites, population growth rates for experimental (artificially established) EAB cohorts dropped from an R_0 value of 16.0 to 4.7 and from 19.4 to 4.6 for wild EAB cohorts. This drop, however, also reflects the progression of ash mortality at these sites. Mortality rates for overstory green ash, white ash and black ash (*Fraxinus nigra* Marshall) trees in much of southeast Michigan exceed 90% and few trees >10 cm dbh remain alive (Burr and McCullough, 2014; Flower et al., 2013; Knight et al., 2013; Klooster et al., 2014). Decreased EAB population growth rates, therefore, reflect the diminished availability of ash phloem for larval development together with increased mortality from natural enemies (Duan et al., 2014).

The cumulative influence of mortality due to native and introduced natural enemies on EAB population trajectories is not yet clear, particularly in areas where the EAB invasion is relatively recent. Populations of EAB in southeast Michigan were established for more than a decade before the first introductions of Asian parasitoids (Gould, 2007) and the first observations of significant larval parasitism by native *Atanycolus* spp. (Liu et al., 2003; Cappaert and McCullough, 2009; Siegert et al., 2014). In states with more recent infestations, however, Asian parasitoids have been introduced within a few years of detection. Whether earlier introduction and establishment of Asian parasitoids will effectively slow the progression of ash mortality in these areas remains to be seen.

Many of the Michigan stands decimated by EAB are characterized by abundant ash regeneration, including seedlings and saplings. Although ash saplings down to 2.5 cm in diameter can be colonized by EAB (Cappaert et al., 2005), trees <10 cm in diameter often escape colonization even during the peak of the EAB invasion wave (Herms et al., 2010; Burr and McCullough, 2014; Klooster et al., 2014; Smith et al., in press 2015). The fate of these young trees will likely determine whether ash persists as a functionally viable component of forest ecosystems in North America. Ash trees must be at least 8-10 cm in diameter before they begin to produce seed (Kennedy, 1990), and frequency of seeding years varies among ash species. Seed crops can be heavy, but losses from unfilled seeds and seed predation (e.g., ash seed weevils [*Lignyodes* spp.]) can be substantial (Solomon et al., 1993), and seeds do not persist in the seed bank over time (Klooster et al., 2014). Ash seedlings are tolerant of shade and may persist in closed canopy stands for several years (Kennedy, 1990). As ash mature, they become increasingly intolerant of shade, and generally require full or nearly full exposure to sun to reach the overstory (Baker, 1949; Gucker, 2005). Canopy gaps resulting from mortality of overstory ash can facilitate recruitment of young ash if gaps are not filled by lateral in-growth of other overstory trees (Bartlett and Remphrey, 1998, Burr and McCullough 2014) or regeneration of competing species (Flower et al., 2013, Smith et al., 2015).

The ability of natural enemies, including native and introduced parasitoids, to prevent young ash from being killed by EAB may play a critical role in the long term survival and persistence of ash across much of North America. Density of EAB in a local area clearly declines as ash trees, particularly large ash trees, are killed. Effects of egg and larval parasitoids on EAB survival may become more pronounced in these areas after the EAB invasion wave goes through and the availability of ash phloem to support EAB has dropped substantially. Complete mortality of EAB life stages is not likely to be necessary; in general, most ash trees are remarkably resilient and tolerate a low level of larval feeding (McCullough et al., 2015). Thus, while ash may no longer function as a dominant overstory species, natural enemies may enable ash trees to persist at some level, providing food and habitat for populations of native insects and mites that are ash specialists (see Chapter 2). The consistent preference for small diameter trees demonstrated by *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), one of the introduced larval parasitoids (Abell et al., 2012; Duan et al., 2013), may be particularly beneficial in this regard.

Given the current and potential impacts of EAB in North America, biological control research and evaluation efforts must continue. Possible effects

of interspecific differences among North American ash in resistance to EAB and the implications of these differences for biological control warrant consideration. Blue ash, *Fraxinus quadrangulata* Michx., growing on fertile sites, for example, appears to be relatively resistant to EAB, while black ash is highly vulnerable to EAB (Tanis and McCullough, 2012; Klooster et al., 2014; Herms and McCullough, 2014). Biological control agents may be more successful at a blue ash site because of its inherent higher resistance to EAB. Conversely, at sites dominated by black ash, introduced parasitoids may be overwhelmed and unable to demonstrate any numerical response to EAB before all or nearly all trees are killed. Evaluating factors associated with relative resistance and vulnerability of different ash species could have important implications for identifying sites where introduced parasitoids are likely to be most effective.

In urban, residential, and even rural areas, effects of combining two or more EAB management tactics should be studied. Systemic insecticides, including products with emamectin benzoate, azadiractin, dinotefuran, or imidacloprid, are translocated in xylem to the canopy branches and foliage (Mota-Sanchez et al., 2009; Tanis et al., 2012). In contrast to cover sprays of insecticides applied to the outer bark, when systemic materials are used, egg parasitoids, such as the introduced *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), will not encounter the insecticide. Larval parasitoids, whether native or introduced, will not attack dead EAB larvae nor will woodpeckers attempt to prey on dead larvae. Using systemic insecticides may therefore offer two benefits: ash trees treated with an effective product are protected from EAB injury and insecticide-reductions in overall EAB density may cause parasitoids or predators to concentrate their efforts on infested but untreated trees. The combination of systemic insecticides and natural enemies could yield an additive effect or perhaps even a synergistic effect if natural enemy reproduction or host searching behavior is enhanced (Barclay and Li, 1991; Suckling et al., 2012). Understanding more about how to optimize the spatial distribution of trees treated with systemic insecticides in a given locality to enhance

parasitism or predation rates could be productive. For example, field studies consistently show girdled ash trees are highly attractive to adult EAB, especially in recently infested sites (McCullough et al., 2009a,b; Mercader et al., 2013). Opportunities may exist to employ girdled or stressed ash to concentrate both EAB and parasitoid populations in selected areas.

Much remains to be learned about native parasitoids, including their host-seeking behavior, cues that elicit parasitism, and the ability of these species to learn and adapt to a new host. Most native parasitoids and insect predators of phloem-feeding beetles are opportunistic habitat specialists, rather than host specialists (Kennedy and McCullough, 2002) and, as such, may be capable of developing on many species, genera, and even families of insects (but see Taylor et al., 2012). Native parasitoids including *Atanycolus* spp., *Phasgonophora sulcata* Westwood (Hymen.: Chalcididae), and *Spathius floridanus* Ashmead (Hymen.: Braconidae), while not well studied, are frequently recovered from trees colonized by native wood- or phloem-borers and in some areas, parasitism of EAB larvae by one or more native species is increasing (Duan et al., 2012).

Many parasitoids are adept at learning combinations of olfactory and visual cues associated with potential host insects and modifying their responses accordingly (Turlings et al., 1993). As an invasive insect population spreads, opportunities for native parasitoids to encounter and adapt to the new invader increase (Vet and Groenewold 1990; Turlings et al., 1993; Grabenweger et al., 2010). Assemblages of native parasitoids may respond and adapt to an invader relatively quickly, but their ability to affect dynamics of an invasive species varies considerably. For example, native generalist parasitoids quickly adapted to light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae) in California and high parasitism rates contributed to population suppression (Wang et al., 2012). In contrast, native parasitoids had little effect on citrus leafminer (*Phyllocnistis citrella* Stainton [Lepidoptera: Gracillariidae]) populations in Spain and responded in a negative density-dependent manner to high pest populations (Vercher et al., 2005). Research on the ability of native parasitoids to adapt and respond to

EAB invasion is needed and could lead to practical and effective tactics to augment parasitism rates by these species.

Considerable research has been conducted to identify semiochemical attractants or visual cues used by native and introduced EAB parasitoids including pheromones for *T. planipennisi*, and *S. agrili* and *S. floridanus* (Bauer et al., 2011, Cossé et al., 2012) and responses of *P. sulcata, S. agrili,* and *S. floridanus* to host kairomones associated with either EAB or ash trees (Roscoe et al., 2011, Johnson et al., 2014). Other research has addressed parasitoid response to visual stimuli including trap colors (Cooperband et al., 2013) and mechano-reception of vibrations by EAB larvae (Ulyshen et al., 2011). Further studies on olfactory, visual, and perhaps vibrational cues used by parasitoids to locate potential hosts may improve EAB biological control. Pre-release conditioning or oviposition manipulation with semiochemicals, for example, might increase parasitoid efficacy. Attractive lures or aerial application of volatile compounds could perhaps provide a means to attract or enhance parasitoid populations.

Given the ongoing expansion of EAB, the economic costs resulting from urban infestations and the still unknown ecological ramifications of this invader for forest ecosystems, it seems clear that an integrated approach is needed to deal with EAB. Understanding and enhancing the collective effects of native and introduced parasitoids and predators on EAB will be crucial if native ash species are to persist in North America.

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