



Can *Spathius galinae* attack emerald ash borer larvae feeding in large ash trees?



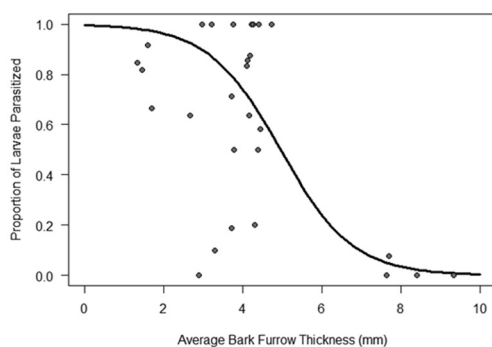
Theresa C. Murphy^{a,*}, Roy G. Van Driesche^{b,1}, Juli R. Gould^{c,2}, Joseph S. Elkinton^{a,b,1}

^a Organismic and Evolutionary Biology, University of Massachusetts Amherst, MA 10003, United States

^b Department of Environmental Conservation, University of Massachusetts Amherst, MA 10003, United States

^c USDA-APHIS-PPQ, Buzzards Bay, MA 02542, United States

GRAPHICAL ABSTRACT



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ABSTRACT

Agrilus planipennis, the emerald ash borer (EAB), is an invasive forest pest decimating North American ash trees. Population-wide management of EAB is focused on biological control through the introduction of four parasitic wasps, including the recently approved larval parasitoid *Spathius galinae*. This species was approved for release in the north-central and northeastern US in 2015 and its long ovipositor (4–5.3 mm) is expected to allow it to reach EAB larvae in larger ash trees with thicker bark, than the only other successfully established larval parasitoid, *Tetrastichus planipennis*. Using experimentally infested logs of varying diameters in the laboratory, we measured the effect of bark thickness on oviposition of *S. galinae* to understand its potential value for controlling EAB in trees of differing diameter and bark thickness. Parasitism by *S. galinae* was highest when bark was thin (< 4 mm) and dropped significantly as bark thickness increased beyond 6.5 mm. We also found that EAB larval feeding on inner bark, reduced the bark thickness directly over the larval galleries by 0.4 mm on average. Our results suggest that *S. galinae* will be able to reach EAB larvae in > 95% of all ash in the northeastern United States. *S. galinae* will likely play a vital role in providing additional control and in supporting ash regeneration in aftermath areas of EAB invasions.

Abbreviations: EAB, emerald ash borer; DBH, diameter at breast height (approximately 1.4 m); USFS, United States Forest Service

* Corresponding author at: 250 Natural Resources Road, Ag. Engineering Rm 115, University of Massachusetts, Amherst, MA 01003, United States.

E-mail addresses: tcmurphy@umass.edu (T.C. Murphy), vandries@cns.umass.edu (R.G. Van Driesche), Juli.R.Gould@aphis.usda.gov (J.R. Gould), Elkinton@ent.umass.edu (J.S. Elkinton).

¹ Address: 160 Holdsworth Way, Dept. of Environmental Conservation, University of Massachusetts, Amherst, MA 01003, United States.

² Address: 1398 West Truck Road, Buzzards Bay, MA 02542, United States.

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1. Introduction

The emerald ash borer (EAB), *Agrilus plannipennis* Fairmaire (Coleoptera: Buprestidae), is one of the most destructive forest invaders in North America (Aukema et al., 2011; Herms and McCullough, 2014). Since its accidental introduction to Michigan in the 1990s, EAB has already spread to 30 states, two Canadian provinces, and has killed over 100 million ash trees, *Fraxinus* spp., (Herms and McCullough, 2014 emeraldashborer.info, 2016). EAB causes severe environmental and economic harm and may result in the functional extinction of several species of *Fraxinus* from North American forests (Aukema et al., 2011; Gandhi and Herms, 2010; Herms and McCullough, 2014). Eradication of the emerald ash borer was attempted when the infestation was first discovered, but was unsuccessful and discontinued (Herms and McCullough, 2014). Due to the logistics and high cost of insecticide applications, use of systematic insecticides in forests is not practical (McCullough and Mercader, 2012). Since 2007, classical biological control (the introduction of non-native natural enemies to regulate an invasive species) has been the dominant management practice for large-scale control of EAB populations (Bauer et al., 2008). As of 2016, the USDA has approved four parasitic wasps for EAB biocontrol: an egg parasitoid (*Oobius agrili* Zhang and Huang) and three larval parasitoids (*Spathius agrili* Yang, *Spathius galinae* Belokobylskij and Strazenac, and *Tetrastichus planipennisi* Yang) (Belokobylskij et al., 2012; Yang et al., 2005; Yang et al., 2006; Zhang et al., 2005).

Tetrastichus planipennisi is the most widely established introduced EAB biocontrol in North America (Bauer et al., 2015); however, Abell et al. (2012) found that this parasitoid species cannot oviposit in larvae in the lower boles of trees with a bark thickness exceeding 3.2 mm (equal to trees with a DBH [diameter at breast height] > 11.2 cm) due to its short ovipositor. This limitation of *T. planipennisi* creates a large refuge for EAB larvae, particularly in stands with more mature, larger ash trees. Data from the United States Forest Service (USFS) show that as of 2014, over 500 million ash trees or 26% of all *Fraxinus* spp. on forested land in the United States northeastern region (Connecticut, Maine, Massachusetts, New Jersey, New York, Pennsylvania, Rhode Island, Vermont) were too large at DBH for *T. planipennisi* (FIDO 2009–2014). Limited *T. planipennisi* parasitism can occur on larger trees, but only where available larvae are under thinner bark (< 3.2 mm) on the upper bole and smaller branches of the tree (Duan, pers. comm.). Another larval parasitoid, *S. agrili*, was also approved for release in 2007 and has a longer ovipositor than *T. planipennisi*, but *S. agrili* has failed to establish north of the 40th parallel (Gould and Duan, 2013; USDA–APHIS/ARS/FS, 2016). With *T. planipennisi* as the only currently established introduced larval parasitoid, many EAB larvae in larger ash trees remain inaccessible to introduced larval parasitoids, highlighting the need for another biocontrol with a longer ovipositor able to attack larvae in larger trees.

Spathius galinae, a third larval parasitoid, which was approved by the USDA for release in 2015, has a longer ovipositor than *T. planipennisi*. The ovipositor of *S. galinae* is 4–5.3 mm in length (Gould and Duan, 2013), while that of *T. planipennisi* is only 2.0–2.5 mm (Duan and Oppel, 2012). Given its longer ovipositor length, *S. galinae* should be able to attack hosts in larger ash trees that *T. planipennisi* (Gould and Duan, 2013). Furthermore, climate matching suggests that there is a better fit between *S. galinae*'s native range in the Russian Far East and that of the north-central and northeastern United States than is true for *S. agrili*, which failed to establish in the north-central region of the United States (Duan and Oppel, 2012; Gould and Duan, 2013). In addition to its long ovipositor and climatic suitability, *S. galinae* is known to cause parasitism rates of up to 63% on EAB in American green ash (*Fraxinus pennsylvanica*) in Russia (Duan et al., 2012). The effect of bark thickness on parasitism has previously been determined using both *T. planipennisi* and *S. galinae*; Wang et al. (2015) found that parasitism rates of *T. planipennisi* on large logs were significantly lower than on small logs. In contrast, there was no significant difference in parasitism

rates for *S. galinae* across log sizes tested (Wang et al., 2015). However, in their experiment the large logs did not exceed 10 cm in diameter. While this diameter is close to the upper limit (11.2 cm) for *T. planipennisi*, we hypothesize that *S. galinae* has a much larger upper size limit than that of *T. planipennisi* due to its longer ovipositor (Abell et al., 2012; Wang et al., 2015). By using larger diameter logs, we examined conditions closer to *S. galinae*'s expected oviposition limit to assess whether a similar change in parasitism is noticeable for *S. galinae* as it approaches its oviposition limit.

As a relatively recently approved agent in the EAB biocontrol program, *Spathius galinae*'s potential impact is poorly known. Our goal was to investigate how oviposition success by *S. galinae* is affected by bark thickness over a wider range of tree sizes. We also wanted to study the effect of EAB larval feeding on bark thickness. EAB larvae feed at the intersection of the inner bark and cambium (Poland and McCullough, 2006), and we wanted to determine if this feeding significantly reduced bark thickness, facilitating parasitism. Knowing *S. galinae*'s oviposition limits would improve EAB management through a greater understanding of the expected impact of *S. galinae* in the field and would assist in future modeling of EAB population dynamics. Researchers can use this information to help choose parasitoid release sites, and managers can possibly choose alternative control methods, such as using trunk injections of pesticides or selected tree removal, to eliminate larvae in large trees deemed inaccessible to larval parasitoids.

2. Materials and methods

Under controlled conditions in the laboratory, white ash, *Fraxinus americana* L., logs were artificially infested with EAB larvae and then introduced to parasitoids in cages after larvae had reached a suitable age for parasitism (3rd or 4th instars) (Abell et al., 2012). After exposure larvae were reared for 2 weeks to allow parasitoids to develop (Duan et al., 2014), and then all logs were debarked to record the fate of the EAB larvae (alive, dead, parasitized), calculate parasitism rates and record bark thicknesses (Duan et al., 2010).

2.1. Log preparation

Three trials of the experiment were run between July 2015 and December 2015. Logs were cut within 3 days of starting each trial. White ash trees were selected in three size classes based on DBH. Small = 3–8 cm DBH, medium = 12–18 cm and large = 25–30 cm. These size ranges were chosen to help ensure a wide range of bark thicknesses. Logs cut from these trees and used in our experiment ranged in diameter from small = 6.4–8.7 cm, to medium = 8.2–15.4 cm, and large = 18.9–32.2 cm. Log lengths and the number of logs were varied among treatments (four to eight small logs, two to four medium logs, and one to two large logs) such that in aggregate each treatment had the same bark surface area available to *S. galinae*. To accommodate equal surface area per treatment, logs were cut anywhere between 10 and 30 cm in length.

2.2. Egg application and EAB larval development

To inoculate logs with EAB, 20 eggs were applied per replicate for all treatments (log or group of logs). EAB eggs were produced and supplied by the USDA-APHIS EAB Rearing Facility (Brighton, MI, USA) and were attached to paper coffee filters. These coffee filters were cut into paper squares with one to three eggs per square. An edge of the filter paper was then glued to the bark, with the eggs facing out. Eggs were then covered with a cotton ball, and the covered eggs secured to the log with breathable quick-drying ribbon, 3.8 mm satin (100% polyester). This egg technique was modified from Abell et al. (2012) because parafilm did not adhere to the large, rough-barked logs that were used in our study. Before egg application, any rough bark surfaces were lightly scraped to create a flat, smooth surface for egg placement.

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