March 2017

FACTORS INFLUENCING IMPACT OF BIOLOGICAL CONTROL AGENTS OF THE EMERALD ASH BORER

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Recommended Citation

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FACTORS INFLUENCING IMPACT OF BIOLOGICAL CONTROL AGENTS OF THE EMERALD ASH BORER

A Thesis Presented

by

THERESA C. MURPHY

Submitted to the Graduate School of
the University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER of SCIENCE

February 2017

Organismic and Evolutionary Biology

FACTORS INFLUENCING IMPACT OF BIOLOGICAL CONTROL AGENTS OF THE EMERALD ASH BORER

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DEDICATION

To my parents and grandparents for their constant and unwavering support.

And to my fellow scientists, “Life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained.”- Marie Curie

ACKNOWLEDGMENTS

I would like to thanks my committee J Gould, J Elkinton R Van Driesche and J Podos, whose help in the field, laboratory, and throughout the writing process has been invaluable. Thank you also to my laboratory colleagues, J Boettner, R Crandall, H Broadley, M Davis, E Mooshian, A Pepi, and J Lombardo for your friendship and support every step of the way.

This research was funded by a grant from the USDA FS (State and Private Forestry) and USDA APHIS.

Thanks to all the colleagues who helped provide advice, input, and support for my research. J. Duan, M. Hickin, B. Kane, K. Gooch, M. Joy, F. Andre, D. Williams, J. Lelito, K. Abell, and L. Bauer for help with project design and data collection. Thank you especially to E. Reynolds and F. Andre (MA DCR) for providing all the logs for my experiments and E. Angell (NY Ag & Markets) for assistance with work done out in New York.

Thank you as well, to all the Elkinton lab and USDA summer techs for their help in setting up and carrying out these experiment, in particular to my BURA students E Lee and M Salhany. R. Hennessey for helping to fix a shelf last-minute for my experiment and A. Roehrig for helping me to take down a tree with only a handsaw. M. Barrett for all your help in quarantine. D. Snider (CRISP) and H. Luedtke (NY Ag & Markets) for your help in New York. Without you this work wouldn't have gotten done.

Thank you to K. McGarigal, C. Sutherland, A. Jordaan, J. Finn, and M. Akresh, for providing statistical advice.

Thanks to all those who hosted me while I was doing fieldwork, J. Gould, A. Roehrig, N. Bruce, K. Wilbur, D. Snider, Z. Williams, M. Teator, and C. Dwyer.

Thank you to D. Burt, S. McMahon, and everyone at the USDA EAB rearing facility in Brighton, Michigan for providing all the eggs, the *S. agrili*, and *T. planipennisi* used my experiments and J. Duan (USDA APHIS) and J. Gould (USDA APHIS) for providing all the *S. galinae* used in my experiments.

And finally, a special thank you to my friends and the OEB community who gave me the friendship, care, and encouragement I needed to stay positive and focused throughout this experience.

Three years is far too short of a time to work among such excellent and admirable people and in the end of I've thanked less than half of you half as well as I should like.

ABSTRACT

FACTORS INFLUENCING IMPACT OF BIOLOGICAL CONTROL AGENTS OF THE EMERALD ASH BORER

FEBRUARY 2017

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Agrilus planipennis, the emerald ash borer (EAB), is a destructive invasive forest pest decimating North American ash trees. Population-wide management of EAB focuses on biological control, with the introduction of four parasitic wasps; one egg parasitoid, *Oobius agrili* and three larval parasitoids- *Spathius galinae, Spathius agrili* and *Tetrastichus planipennisi.* This thesis examines some of the factors influencing the establishment of these larval biocontrol agents. Chapter 1 examines the relationship between woodpeckers and the parasitoids *S. agrili* and *T. planipennisi.* Both woodpeckers and these parasitoids attack the larval stage of EAB, which means their impacts overlap and potentially interact. To examine this relationship, I established parasitized larvae on ash trees and then used screening to exclude woodpeckers from some sections of the tree. Results show that while there is no evidence of discriminatory feeding for or against parasitized larvae, the presence of parasitized larvae changes woodpecker feeding behavior at a stand-level. I hypothesize that this change is due to these larval parasitoids being a low-food reward and that parasitism contributes to a change and decrease in patch quality, causing woodpeckers to quit foraging sooner than usual.

My second chapter focuses on *Spathius galinae,* which was recently approved for release in the north central and northeastern US in 2015, to provide additional population control. *Spathius galinae's* long ovipositor (4-5.3mm) is theoretically expected to help target EAB in ash with larger diameters and bark thicknesses. Using experimentally infested logs of varying thicknesses in the laboratory I tested the limits and preferences for oviposition of *S. galinae*, to understand its potential impact on EAB. My results demonstrated that although parasitism by *S. galinae* drops significantly when bark thickness reaches 8 mm, this prevents *S. galinae* only from reaching EAB larvae in my largest ash trees (<5% of ash in the Northeast). Given the natural variations in bark thickness and the 0.4 mm thinning effect of EAB on inner bark thickness, I am confident that *S. galinae* will play a vital role in providing additional control and in supporting ash regeneration in aftermath areas of EAB invasions.

Keywords: mortality agents; density-dependence; bark thickness; oviposition; parasitoids; woodpeckers

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CHAPTER 1

INTERACTIONS BETWEEN WOODPECKER PREDATION AND PARASITOIDS OF THE EMERALD ASH BORER

1.1 Introduction

Since its discovery in Michigan in 2002, the emerald ash borer (EAB), *Agrilus plannipennis* Fairmaire (Coleoptera: Buprestidae), has spread to 30 states and two Canadian provinces in the U.S., killing millions of native ash, *Fraxinus* sp., trees (Herms & McCullough, 2014). It is now considered one of the most destructive forest pests to ever reach North America (McCullough, 2013) and is expected to cost over 10 billion dollars to treat, remove, and replace infested ash (Kovacs et al., 2010). Eradication using mechanical control was tried unsuccessfully while systematic insecticides are economically prohibitively at a forest scale (Bauer et al., 2008; Herms & McCullough, 2014). Management of EAB populations is focused on classical biological control, the introduction of non-native natural enemies to help regulate the population, as the most feasible population-level tool currently available to control EAB densities (Bauer et al., 2008; Herms & McCullough, 2014). To date, EAB biocontrol has introduced four parasitic wasps: three larval parasitoids (*Spathius agrili* Yang, *Spathius galinae* Belokobylskij and Strazenac, and *Tetrastichus planipennisi* Yang) and an egg parasitoid (*Oobius agrili* Zhang and Huang) (Belokobylskij et al., 2012; Yang et al., 2005; Yang et al. 2006; Zhang et al., 2005).

Evaluating the impact of natural enemies to control a pest requires detailed quantification of the mortality factors in the system. Approaches to such studies include life tables (used for organisms like insects that have distinct life stage) or matrix models (used mainly for organisms like plants that lack clearly defined life stages), to understand the population dynamics of the study species (Bellows et al., 1992; Caswell, 2001). Proper construction and interpretation of life tables or matrix models requires careful measurement of the rates of all important mortality factors in the study species' life system. This process can be complicated by interactions among competing mortality factors that affect a common stage and thus act contemporaneously. Accurately quantifying the impacts of mortality factors that act contemporaneously is challenging and depends on whether attack rates by one agent influence the observable attack rates of other agents (Elkinton et al., 1992; Royama, 1981).

Experimental studies, such as the one presented here, are essential to understanding the true impacts of competing mortality agents (Campbell & Torgersen, 1983; Roland, 1990). Particularly relevant is whether the observable effectiveness of introduced biological controls will be affected by other factors already in the system. In North America, most emerald ash borer populations suitable for parasitoid release will likely experience significant woodpecker feeding, particularly in the winter (Jennings et al., 2015; USDA–APHIS/ARS/FS., 2016). Woodpecker predation of EAB larvae in North America can be very high (30-95%) in infested forests, and can reduce larval densities in trees by 33.5% - 88.2% (Cappaert et al., 2005; Jennings et al., 2015). Woodpeckers may also consume the larvae, pupae or even adults of the introduced larval parasitoids. Potential interactions between these two mortality factors will be useful for the management of EAB (Jennings et al., 2013). I expect hairy woodpeckers (*Picoides villosus* L) and downy woodpeckers (*Picoides pubescens* L) to be the most common predators of EAB in the study areas (Flower et al., 2014; Jennings et al., 2015). Predation from red-bellied woodpeckers (*Melanerpes carolinus* Linnaeus) may also be important, as they have also been shown to feed on EAB larvae and have increased in abundance in the past few decades in both New York and Massachusetts (Shackelford et al., 2000).

Selective predation pressure on parasitized versus non-parasitized larvae by generalist predators has been important in other invasive species systems. The marginal attack rate is the proportion of individuals attacked by an agent, if that agent acted alone, often called the true underlying rate in the system (Buonaccorsi & Elkinton, 1990; Elkinton et al., 1992; Royama, 1981). This can differ from the apparent attack rate that researchers observe, if two factors, such as predation and parasitism, overlap. In a study of predation by generalist predators on winter moth pupae, *Operopherta brumata* L., and pupae parasitized by the introduced biological control fly, *Cyzenis albicans* Fallen, researchers found that predators preferentially attacked unparastized pupae, instead of parasitized pupae (Roland, 1990). Roland concluded that this preference, which increases the apparent parasitism rates of winter moth, was likely a principal factor facilitating the success of the introduced biological control fly, *C. albicans*, contributing to its ability to regulate outbreaks of winter moth (Roland, 1990). In populations of the forest tent caterpillar, *Malacosoma disstria* Hübner, researchers have evidence suggesting generalist predators do not discriminate between parasitized and non-parasitized larvae of *M. disstria*, but do preferentially avoid parasitized pupae (Glasgow, 2006; Nixon & Roland, 2012). This avoidance results in higher apparent parasitism rates of pupae exposed to predators, which like in the winter moth system, augments top-down control (Glasgow, 2006; Nixon & Roland, 2012). Little work has been done to look at predator/parasitoid relationships in the EAB system. Jennings et al. (2013) found evidence of a significant decrease in parasitism when woodpecker predation was also present compared to when woodpeckers were excluded. A small but significant difference, a decrease in parasitism rate, was seen between open trees (1.2% parasitism) and caged or woodpecker excluded trees (3% parasitism) (Jennings et al., 2013). However, this study only compared total fates and not apparent parasitism or interactions between the two fates. Anecdotal evidence of half-eaten broods of parasitized larvae (personal observation) has led us to hypothesize that parasitized larvae may be less preferred by woodpeckers, but to date this has not be investigated experimentally.

Oviposition by my study parasitoids, *T. planipennisi* and *S. agrili*, occurs from late spring through fall, after beetle larvae have reached a suitable size for attack (3rd instar to 4th) (Jennings et al., 2013). The immature stages of these parasitoids develop over the winter months and emerge in the spring. I wanted to explore the relationship between woodpeckers and both parasitoids because their differences in lifecycle, *T. planipennisi* an internal endoparasitoid and *S. agrili* an external ectoparasitoid which forms overwintering cocoons, might lead woodpeckers to interact with them differently. Although woodpecker predation on EAB larvae can occur year-round, most predation occurs from September to February, which coincides with crucial overwintering of parasitoid populations inside EAB galleries (Jennings et al., 2013). Due to their contemporaneous nature and the critical overlap in timing of parasitoid overwintering and woodpecker predation, there is strong potential for an interaction between EAB mortality due to woodpeckers and introduced parasitoids.

This study was designed to quantify the relationship between woodpecker predation and parasitism of EAB larvae, to understand if these mortality factors are discriminatory or non-discriminatory, and to determine if woodpeckers demonstrate any preference or avoidance of parasitized EAB larvae. If woodpeckers avoid parasitized EAB¸ then the benefits of biological control from the combined action of parasitoids and predators may be enhanced, and the potential of both agents working together to lower EAB population densities would be greater (Nixon & Roland, 2012; Roland, 1990). Alternatively, if woodpeckers preferred parasitized larvae and caused considerable mortality, this predation would lessen the effectiveness of parasitoids as control agents, by hampering their ability to increase in population and suppress the pest population (Tostowaryk, 1971). Regardless of the type of relationship, untangling these two mortality factors should be useful for managers constructing life tables and should provide important context information for researchers working on the most effective management approach of EAB.

1.2 Methods

1.2.1 ***Site selection.*** The study was conducted at seven forest sites, each with a high percentage of ash (at least 30%) and active EAB infestations, in either Massachusetts or New York (Table 1).

Table 1 Study site locations and various characteristics from MapBioControl.

Site	GPS Coordinates	Date of initial EAB discovery	% Ash[1]	Site EAB Density Index[2]	Bole EAB Density (larvae/m)[3]
N. Andover, MA	42.71199, -71.11691	2013	50	Medium	33.0
Dalton, MA	42.417055, -73.19155	2012	75	Medium	23.6
Pittsfield, MA	42.42245, -73.26569	2012	34	N/A	23.6
Cementon, NY	42.14616, -73.91954	2011	50	High	42.8
Catskill, NY	42.18221, -73.90839	2011	57	High	72.6
Saugerties, NY	42.12109, -73.94752	2011	50	High	95.4
Lake Katrine, NY	41.99242, -73.99795	2011	50	High	30.1

[1] Percentage ash by mature tree count.

[2] Low Density: EAB present but difficult to find. Nearly 100% of ash trees are healthy. Medium Density: Trees are beginning to show signs of EAB infestation (epicormic shoots, woodpecks, bark splits, emergence holes) but >75% of the trees are healthy and show no signs of EAB. High Density: >25% of the trees show signs of EAB infestation.

[3] Average density of mature (>2nd instar larvae) in the peeled tree boles.

1.2.2 ***Study design.*** Treatments were established by caged sections of tree boles that had either been inoculated earlier with EAB eggs to obtain larvae, or where larvae were presumed to exist because of high density at the site. Experimental units consisted of two one-meter caged bole sections on select trees, setup as described below. The study involved three treatments: (1) EAB larvae only (control), (2) EAB larvae exposed to adult *S. agrili*, and (3) EAB larvae exposed to adult *T. plannipennisi*. Further, each treatment had subplots (different caged bole sections on the same tree) that either were exposed to woodpecker predation after exposure to parasitism or where woodpecker predation was prevented (Fig. 1). This was accomplished by covering the bark of the section with aluminum screening to prevent woodpecker attack (Fig. 2B).

1.2.3 ***Tree selection.*** At each site, four trees per parasitoid treatment were selected (twelve trees per site and 84 trees in total). *Tetrastichus planipennisi* cannot attack EAB on the lower trunk of trees with a DBH >11 cm (Abell et al., 2012). Therefore, to accommodate this species in the treatment scheme, at least six trees were selected per site (four for exposure of EAB to *T. planipennisi* and two to act as controls) that were between 6 and 11 cm DBH. Trees selected for controls, (EAB-only), ranged from 6 to 20 cm DBH. Trees selected for EAB parasitized by *S. agrili* ranged from 11 to 18 cm DBH (Abell et al., 2012). At the New York sites, trees were not inoculated with eggs but instead I selected naturally infested trees based on signs of EAB infestation (epicormic shoots, current year woodpecker predation, bark splits, or poor crown condition) (Gould et al., 2012). At the Massachusetts sites the EAB infestations were more recent, and EAB density was much lower than in New York. Consequently, to increase EAB densities I inoculated the study trees with EAB eggs (see below).

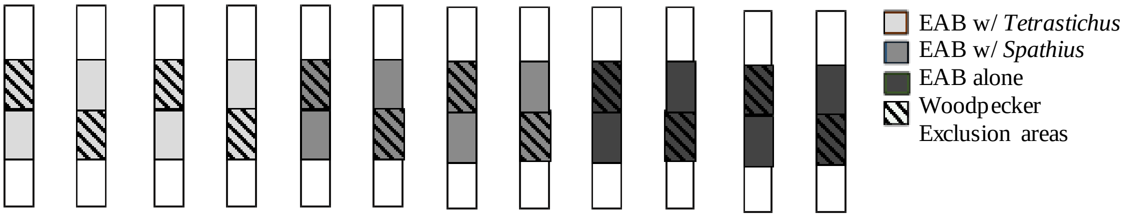

Figure 1 Twelve trees (rectangles) representing one site with four trees for each parasitoid treatment and subplots of woodpecker access and exclusion for each tree.

Figure 2 A) Cage set-up for conducting controlled releases of parasitoids to ensure parasitism in the treatment trees B) Aluminum wire window screening that prevents woodpecker predation but still allows woodpecker to walk freely up and down the tree.

1.2.4 *EAB egg inoculation.* To increase larval numbers in study trees at the sites in Massachusetts with low-density EAB infestations, EAB eggs were applied to trees at three sites (Pittsfield, Dalton, and North Andover) following a protocol outlined in Abell et al. (2012). EAB eggs were provided by the APHIS EAB Rearing Facility in Brighton, Michigan, USA on paper coffee filters. The filters were cut into strips of 1-3 EAB eggs, and then applied in two bands, at 25 cm and 75 cm from the bottom of the experimental 1-meter section of bole. Egg numbers ranged from 25 to 55 and were altered to infest each 1-meter bole section with the same density of eggs, 100 eggs/ m^2 of surface area. Hatch success varied from 12 to 83% but averaged 49% success. Eggs were applied to trees on June 6th,11th, and 12th of 2014 to increase the number of larvae that would be late instars (suitable for parasitoid oviposition) by late summer.

1.2.5 *Girdling.* In March of 2015 trees at the Dalton and Pittsfield sites were girdled at a height of 3 m to promote faster larval development (Noel, 1970; Tluczek et al., 2011). This was done after larvae within these trees failed to grow beyond first and second instars by the end of the summer in 2014. This slow development likely was caused by the relatively healthy state of the trees at these locations (Herms & McCullough, 2014).

1.2.6 *Construction of tree cages for parasitoid treatments.* When mature EAB larvae were present in the study trees, in August of 2014 and 2015, 1 m long cages (two per tree) were constructed over the infested trunks of trees selected for use. EAB in the caged trunk sections were then exposed to parasitoids by inserting females parasitoids (*S. agrili* and *T. plannipennisi*) into the cages. Cages were placed 25 cm from the base of the tree and an additional 25 cm was left between the first and second cage on the trunk. Cage construction followed Abell et al. (2012) with some modifications. The cages were made of extra-fine gauge No-See-Um mesh fabric (OnlineFabricStore, West Springfield, MA) stretched around the trunk and held off it by a wood and wire frame. The wooden frame (l x w x h, 75 x 7.6 x 2.5 cm) was attached vertically along one side of the tree over which the fabric was stretched. Wire was wrapped around the top and the bottom of the wooden frame and kept taut by nails secured into the bole to keep the mesh expanded off the tree. Caulk and foam were used to attach the cage to the tree trunk at the top and bottom of the one-meter bole section. This design enclosed the parasitoids but allowed them to move freely over the bark. The mesh fabric was secured around the tree with duct tape and staples, and then zip-ties were cinched onto the foam to provide a secure mesh cage that would prevent parasitoid escape (Fig. 2A).

1.2.7 *Parasitoid releases.* Additional suitable study trees were flagged, and in Massachusetts inoculated with eggs, to allow such units to be debarked to check for larval development. Parasitoid releases into the cages were made once larvae were found to be in a suitable stage for parasitism (3rd and 4th instars) based on debarking these extra trees (Abell et al., 2012). Naive adult parasitoids, less than a week old and mated, were provided by the USDA-APHIS EAB Rearing Facility in Brighton, MI, USA.

In 2014, at the North Andover, Massachusetts site, 200 females of *T. planipennisi* were released per cage every two weeks, starting on August 22nd and continuing until 800 females were released. Due to the limited number of *S. agrili* that were available, only one release of 21 *S. agrili* females per cage was made.

In 2015, at the other 6 sites, cages were inoculated with 150 females per cage of *T. planipennisi* and 56 females per cage of *S. agrili* per release. Inoculations were started on August 6th and were divided into two inoculations separated by a two-week interval.

Numbers between species varied based on the different efficacy rates of host location in the two species (Ulyshen et al., 2010). Parasitoids were provided with honey that was streaked onto the outsides of the cages and cages were misted with water once a week for four weeks after each release to increase parasitoid survival and oviposition.

1.2.8 *Woodpecker exclusion.* One month after the last parasitoid releases, all cages were removed and aluminum screening was placed over the bark of the formerly caged bole sections of experimental trees that were designated for woodpecker exclusion. Screening was stapled around the boles sections as the cages were removed. The control bole-sections (one per tree), to which woodpecker access was permitted, were left exposed to woodpecker predation throughout the winter (Fig. 2B). To ensure that there was no effect of height along the tree trunk, the exposed bole was assigned randomly to the top for half of the trees and to the bottom for the other half of the trees within each treatment.

Starting in 2015, window screening was added to both sections of the control trees at the same time parasitoid cages were built around the experimental trees. This was to prevent woodpeckers from feeding on control trees while all experimental trees were protected from woodpeckers by the parasitoid cages. One control tree in North Andover experienced limited woodpecker predation in the fall of 2014 while parasitoid cages were in place, prompting this measure to be implemented. Screening was removed from one section of each control when screening was applied to one section of all experimental trees, so that all trees overwintered with one exposed section of tree bole and one protected section.

1.2.9 ***Tree debarking to assess parasitism rates among treatments.*** On 16 March 2015, trees were cut down at the North Andover, MA study site, and on 14 March and 19 April, 2016, trees were cut down in New York and Dalton and Pittsfield, MA sites, respectively. Once trees were cut down, the bark within all caged areas was removed to determine the fate of each EAB larva as alive, eaten by a woodpecker, diseased, parasitized, or died from unknown causes (Duan et al., 2010). For cases in which woodpeckers had successfully removed an EAB larva, careful examination was made of the EAB gallery for any evidence of parasitoids (*Spathius* cocoons, *Tetrastichus* larvae or their meconia[1] or exit holes?).

1.2.10 *Statistical methods.* I used generalized linear mixed-effects models (GLMMS) with binomial error distribution and logit link to analyze (a) the importance of woodpecker exclusion (presence of screening) on the proportion of larvae detected as parasitized, based on those larvae that had not been consumed by woodpeckers. Separate analyses were conducted for each of the two parasitoid species. All analyses were conducted in R version 3.2.5 (R Core Team, 2013).

When determining parasitism rates, any larvae removed by predation were not included in the denominator n, (P/n) X 100 = % Par), since their fate before predation could not be determined. Bole diameter, woodpecker exclusion (presence/absence of screening), bole position (top/bottom), and larval density/m ^{2} within each bole were included as independent variables in the initial models for both species. Site and tree identity-within-site were incorporated as random effects.

Another GLMM model was run across all treatments to compare the intensity of woodpecker predation rates among the three treatments (*S. agrili*, *T. planipennisi*, and no-parasitism control). In addition to treatment as the main independent factor, I also examined the effects on woodpecker predation rates of bole diameter, bole position (top vs. bottom), and larval density (per m^2) within each bole, which were initially included as important independent variables in the model. In these analyses, site was incorporated as

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[1] Fecal mass released at pupation.

a random effect. Three additional models were run, separating each treatment, to look more closely at how percentage predation in each treatment related to larval density.

Models were then reduced using drop1 (R package) to improve the explanatory power of the model using Akaike's Information Criterion (AIC). Any non-significant factors, besides woodpecker exclusion, were dropped until the model had the lowest AIC. Across all models, larvae younger than the 3rd instar were excluded from the analyses, as they would not have been susceptible to parasitism or predation. Additionally, to help with model convergence, bole diameter and larval density were standardized to z-scores before analysis. A Z-score is defined as $Z = (Y-M_y)/S_y$, where Y is the original score, M_y is the mean of the sample and S_y is the standard deviation of the sample. It centers and normalizes the distribution, allowing for comparison of values that come from different distributions (Abdi, 2007).

1.3 Results

1.3.1 *Effects of woodpecker exclusion on parasitism.* Rates of parasitism were comparable amongst larvae not removed by woodpeckers in boles exposed to woodpeckers versus areas protected from woodpeckers. Out of 650 EAB larval fates recorded in *T. planipennisi* boles, the average percentage of parasitized larvae remaining after predation per bole was 14% in woodpecker exposed boles vs 17% in boles where woodpeckers were excluded (*P*= 0.69; Fig. 3). Out of 1236 EAB larval fates recorded in *S. agrili* boles, the mean percentage of parasitized larvae remaining after predation per bole was 22% vs. 25% in woodpecker exposed vs excluded trees (*P*= 0.30; Fig. 3). Both the original and reduced GLMM analyses found that excluding woodpeckers had no significant effect on parasitism by either species (Fig. 3, Table 2 & 3). For *S. agrili*, a reduced model included just two factors: woodpecker exclusion and the position of the exclusion screening along the tree (top or bottom), ($P< 0.01$; Table 2). For *T. planipennisi* a reduced model dropped all factors except woodpecker exclusion and bole diameter ($P< 0.01$; Table 3).

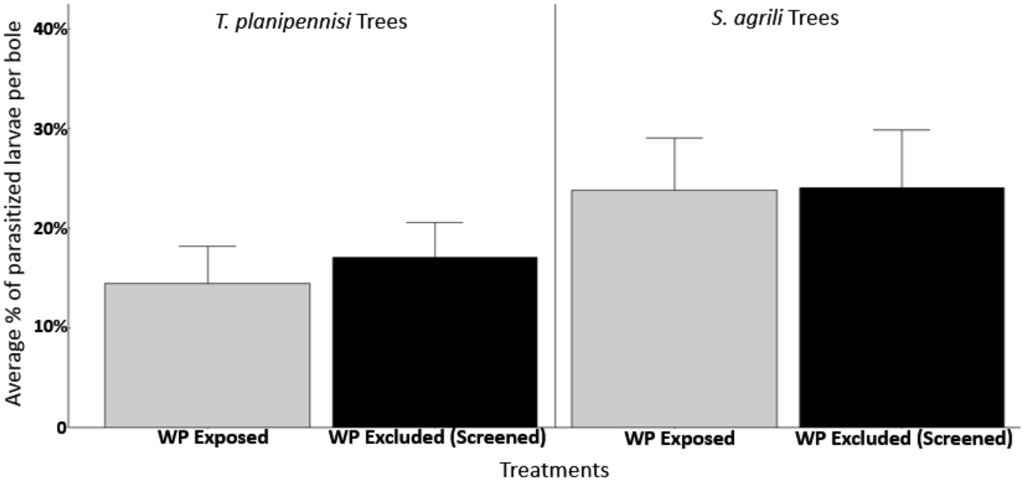

Figure 3 Mean percentage parasitism of remaining *Agrilus planipennis* larvae (± 1 SE) of treatment boles exposed to or excluded from woodpecker predation (screened). Full and reduced logistic regression models of both parasitoid treatments (*Spathius agrili* and *Tetrastichus planipennisi*) found that these percentages were not significantly different from one another (Table 2 & 3).

Table 2 Parameter estimates and statistical significance of various fixed and random effects on proportion of *Agrilus planipennis* larvae parasitized, amongst *Spathius agrili* treatment trees as determined by generalized linear mixed models (GLMMs) with binomial error distribution and logit link.

Full Model AIC=258.6

Fixed Effects	**Estimate**	**SE**	**z-value**	***P***
(Intercept)	-1.9066	0.4008	-4.757	1.97E-06***
Bole Diameter	-0.217	0.2556	-0.849	0.396
Woodpecker exclusion	-0.188	0.1818	-1.034	0.301
Position of exclusion area along tree (top)	-0.1317	0.1856	-0.709	0.478
Larval Density	0.3085	0.2275	1.356	0.175
Random Effects		**Variance**	**Std. Dev.**	
tree:site	(Intercept)	2.58	1.606	
Site	(Intercept)	0	0	

Reduced Model AIC=256.4

Fixed Effects	**Estimate**	**SE**	**z-value**	***P***
(Intercept)	-2.0105	0.4017	-5.005	5.59E-07***

Woodpeckers exclusion	-0.1372	0.1854	-0.74	0.4593
Position of exclusion area along tree (top)	0.4209	0.1856	2.268	0.0233*
Random Effects		**Variance**	**Std. Dev.**	
tree:site	(Intercept)	2.815	1.678	
Site	(Intercept)	1.155E-09	3.398E-05	

Table 3 Parameter estimates and statistical significance of various fixed and random effects on proportion of *Agrilus planipennis* larvae parasitized, amongst *Tetrastichus planipennisi* treatment trees as determined by generalized linear mixed models (GLMMs) with binomial error distribution and logit link.

Full Model AIC= 168.8

Fixed Effects	**Estimate**	**SE**	**z-value**	***P***
(Intercept)	-1.84057	0.49917	-3.687	0.000227***
Bole Diameter	-0.80684	0.31423	-2.568	0.010239*
Woodpeckers excluded	0.10984	0.27204	0.404	0.686385
Position of exclusion area along tree (top)	-0.25685	0.30136	-0.852	0.394039
Larval Density	-0.06821	0.20067	-0.34	0.733935
Random Effects		**Variance**	**Std. Dev.**	
tree:site	(Intercept)	0.3182	0.5641	
Site	(Intercept)	0.9635	0.9816	

Reduced Model AIC=165.5

Fixed Effects	**Estimate**	**SE**	**z-value**	***P***
(Intercept)	-2.0256	0.4298	-4.713	2.44e-06***
Bole Diameter	-0.6103	0.2318	-2.633	0.00846*
Woodpeckers excluded	0.1832	0.2361	0.776	0.43785
Random Effects		**Variance**	**Std. Dev.**	
tree:site	(Intercept)	0.4311	0.6566	
Site	(Intercept)	0.7886	0.8880	

1.3.2 *Effect of treatment on overall woodpecker predation.* Larval fates were recorded in control trees exposed to woodpeckers and out of 124 fates recorded, 38% per bole were depredated by woodpeckers. Out of 382 larvae in *T. planipennisi* treatment trees exposed to woodpeckers, 33% on average per bole were depredated, and out of 687 larvae in *S. agrili* treatment trees, 14% on average per bole were depredated (Fig. 4). The GLMM of woodpecker predation found that *S. agrili* and *T. planipennisi* trees had significantly lower predation (*P* <0.001) than did control trees (Table 4; Fig. 4). *Spathius agrili* trees had the lowest predation rates, followed by *T. planipennisi*, followed by controls. This model also found that diameter and bole position were significant predictors of predation, with predation generally being higher further up on the tree and lower as bole diameter increased, across a range of 5 to 20 cm diameter. Only one model is shown because the full model was also the model with the lowest AIC value.

1.3.3 *Effect by treatment, of larval density on woodpecker predation.* In separate models run on each treatment, control trees showed that predation was significantly positively correlated with larval density (*P* <0.001), predation on *T. planipennisi* treatment trees was significantly but negatively correlated with larval density (*P* <0.001), and predation on *S. agrili* treatment trees parasitism was not correlated with larval density (*P* =0.35) (Table 4; Fig. 5).

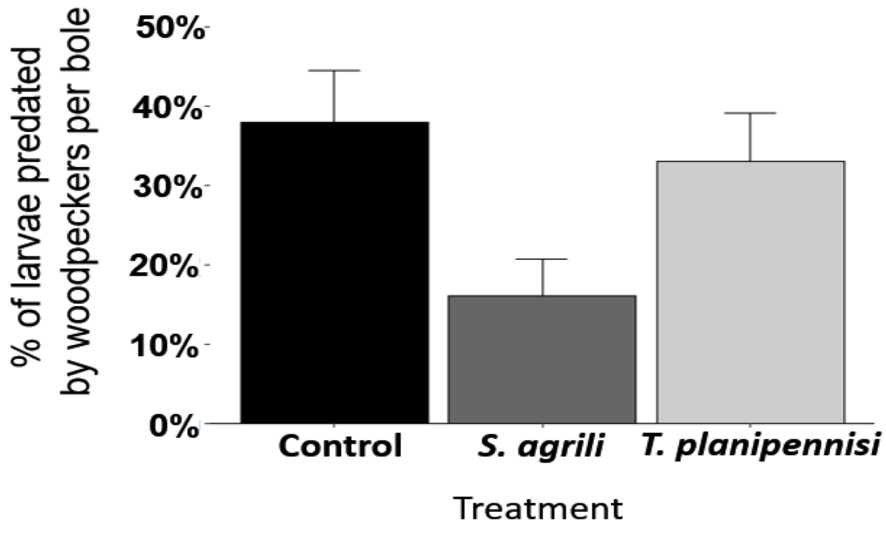

Figure 4 Mean percentage of mature *Agrilus planipennis* larvae predated by woodpeckers (± 1 SE), separated by treatment (Control (EAB-only), *Spathius agrili,* and *Tetrastichus planipennisi*). A generalized linear mixed model of these results (Table 4) confirms that both parasitoid treatments *S. agrili* and *T. planipennisi* had significantly lower predation rates than the control trees.

Table 4 Parameter estimates and statistical significance of various fixed and random effects on proportion of *Agrilus planipennis* larvae predated, amongst the exposed boles of all treatments (*Spathius agrili*, *Tetrastichusplanipennisi*, control (EAB-only)) determined by generalized linear mixed models (GLMMs) with binomial error distribution and logit link.

Full Model - All Treatments AIC=594.6

Fixed Effects	**Estimate**	**SE**	**z-value**	***P***
(Intercept)	-0.59735	0.23486	-2.543	0.010978*
Treatment *S. agrili*	-1.19551	0.2123	-5.631	0.0000000179***
Treatment *T. planipennisi*	-0.7434	0.19537	-3.805	0.000142***
Bole Diameter	-0.38286	0.11306	-3.386	0.000708***
Position of woodpecker exclusion along tree (top)	0.63852	0.16224	3.936	0.000083***
Larval Density/m^2	-0.14237	0.07792	-1.827	0.067678.
Random Effects		**Variance**	**Std. Dev.**	
Site	(Intercept)	0.1781	0.422	

Full Model- Control Treatment, AIC=145.6

Fixed Effects	**Estimate**	**SE**	**z-value**	***P***
(Intercept)	-0.24707	0.22846	-1.081	0.279
Bole Diameter	0.09895	0.17569	0.563	0.573
Position of woodpecker exclusion along tree (top)	-0.25175	0.28926	-0.87	0.384
Larval Density/m^2	1.2983	0.25458	5.1	3.40E-07***
Random Effects		**Variance**	**Std. Dev.**	
Site	(Intercept)	0	0	

Full Model- *T. planipennisi* Treatment, AIC= 158.8

Fixed Effects	**Estimate**	**SE**	**z-value**	***P***
(Intercept)	-2.1401	0.4265	-5.017	5.24E-07***
Bole Diameter	-1.1079	0.3436	-3.224	0.00126**
Position of woodpecker exclusion along tree (top)	1.3108	0.3014	4.349	1.37E-05***
Larval Density/m^2	-0.8629	0.2042	-4.226	2.38E-05***
Random Effects		**Variance**	**Std. Dev.**	
Site	(Intercept)	0.4555	0.6749	

Full Model- *S. agrili* Treatment, AIC=167.2

Fixed Effects	**Estimate**	**SE**	**z-value**	***P***
(Intercept)	-3.1139	0.8075	-3.856	0.000115***
Bole Diameter	0.9481	0.415	2.285	0.022324*
Position of woodpecker exclusion along tree (top)	0.7862	0.3679	2.137	0.032581*
Larval Density/m^2	0.1176	0.1259	0.934	0.35054
Random Effects		**Variance**	**Std. Dev.**	
Site	(Intercept)	2.701	1.643	

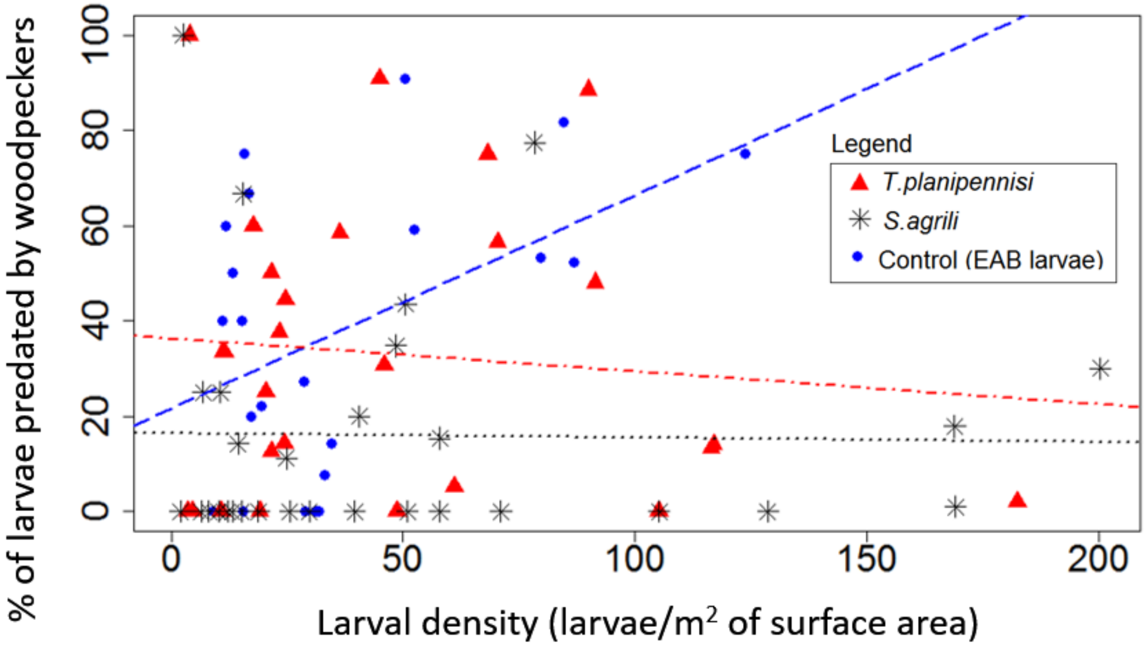

Figure 5 Larval density of EAB within the bole section versus proportion of *Agrilus planipennis* larvae predated. Separated by treatment (control (EAB-only), *Spathius agrili*, and *Tetrastichus planipennisi*), with linear lines of best fit for each treatment. Table 4 show the results of separate generalized linear mixed models run on each treatment, which confirm the regression in this figure. Control shows a significant positive correlation to larval density, *T. planipennisi* shows a significant negative correlation and *S. agrili* is not correlated to larval density.

1.4 Discussion

Our results confirmed that, when feeding on a given tree, woodpeckers did not or could not discriminate between parasitized and non-parasitized larvae. However, at a stand-level, woodpeckers fed more extensively on trees where they did not encounter parasitized larvae. I consider this latter result to be an effect of the different treatments and not a result of tree condition. Previous work has shown that woodpecker predation of EAB larvae is weakly related to site-level variables such as time since infestation and site crown condition and moderately related to tree-level variables like EAB density (Jennings et al., 2013; Lindell et al., 2008). Also, there is evidence that woodpeckers prefer trees in poorer condition with heavier infestations (Jennings et al., 2013). The model accounted for site, which varied across different infestation levels, and EAB densities, but still found a significant difference in woodpecker predation rates across treatments. Thus, I concluded that the difference in predation can only be explained by the difference in parasitism amongst the treatments and not by other site or tree factors.

I hypothesize that woodpeckers may stop foraging more quickly on trees where they encounter parasitized larvae, because they are a lower food reward for woodpeckers. Previous studies have suggested that woodpeckers feeding on ash use a quitting harvest rate foraging strategy, where they choose to stop feeding on patch, or tree, when the foraging costs outweigh the benefits (Brown, 1988; Flower et al., 2014). If parasitized larvae are a lower food reward, the act of parasitism would reduce the patch quality of tree, and if a tree has a reduced patch quality then woodpeckers are likely to quit feeding sooner.

Hairy, downy, and red-bellied woodpeckers feed on ash by creating a single hole through which they stick a barbed tongue to pull out their food (Bent, 1939). This means that the larvae of parasitoids are less accessible than intact EAB larvae. For a woodpecker to extract parasitoid larvae, it would require multiple separate extractions versus one extraction to pull out a large EAB larva. This low-food reward hypothesis is supported by the inherent biology of parasitoids, which have less available energy than EAB larvae because they are of a higher trophic level, and by the differing biology of the two study-species (Yang et al., 2005; Yang et al., 2006). *Spathius agrili* is an external parasitoid, so it's harder for a woodpecker to remove a parasitized EAB larvae as soon as the *S. agrili* larva start feeding. Additionally, *S. agrili* larvae spin silk cocoons, further reducing food quality for the woodpeckers. Biologically, this would make *S. agrili* the least rewarding food source. In support of the low-food reward hypothesis, *S. agrili* treatment trees experienced the lowest predation rates. *Tetrastichus planipennisi* is an internal parasitoid for seven days at 25°C (Duan et al., 2011) a newly parasitized larva indistinguishable to woodpeckers from non-parasitized larva while the parasitoids are still internal. Even once *T. planipennisi* larvae emerge from the EAB, they do not reduce food quality by spinning cocoons. Biologically, *T. planipennisi* would be an intermediate food source and again, in support of my hypothesis, I did observe *T. planipennisi* treatment trees as having intermediate predations rates that were only moderately lower than EAB-only control trees.

Also in support of my low-food reward hypothesis, several recorded incidences of woodpecker predation on *S. agrili* or *T. planipennisi* broods showed that woodpeckers only extracted parts of attacked broods, which provide less food than non-parasitized larvae (Fig. 6A&B). In total, across the 2930 larvae examined in my analysis, seven such interactions were recorded spread over four of the seven sites. I also note that unparasitized larvae attacked by woodpeckers were never only partially consumed (Fig. 6C). This type of interaction, where woodpeckers do not completely consume a *S. agrili* or *T. planipennisi* brood, may also contribute to why these parasitoids are a lower food reward for foraging woodpeckers.

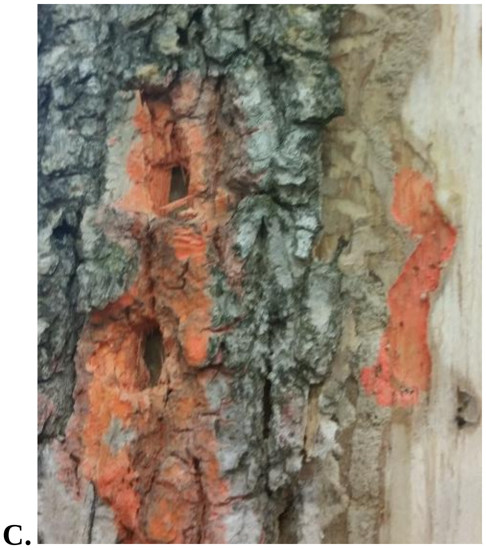

Figure 6 A. A brood of *Tetrastichus planipennisi* that was attacked by a woodpecker. B. A brood of *Spathius agrili* that was attacked by a woodpecker. In both cases part of the brood is still intact. C. A partially peeled log showing two woodpecker holes from the outside and one peeled woodpecker hole with the larval gallery and missing larvae due to predation, exposed. Orange paint used to emphasize woodpecker damage.

Whether the presence of parasitized larvae reduces, enhances, or has no effect on overall predation by woodpeckers on EAB is not clear from the results. In my experimental plots, woodpeckers could choose between parasitized and non-parasitized trees, making any tendency toward preference or aversion easier to detect. Once parasitoids have become established in a natural EAB population, parasitized larvae might occur in most or all trees. Results from Michigan found 94% of trees with at least one brood of *T. planipennisi* (Duan et al., 2013). If established parasitoids do not aggregate and instead spread out, it could be that the presence of parasitoids reduces overall woodpecker attack rates on EAB in the stand. Then, the two mortality agents would be antagonistic to one another. Alternatively, if parasitoids attacks are aggregated, woodpeckers might be able to seek out trees with lower parasitism and attack those preferentially, as they did in my study. In that case, the two mortality agents might have a synergistic effect on overall EAB mortality in the stand.

The relationship I see between these two mortality factors, predation and parasitism, will likely depend on how they interact overall with EAB populations and whether they show density dependent or inverse-density dependent parasitism. In theory density dependence, a positive correlation between parasitism and host density, should be common in host-parasitoid interactions, although direct density dependence has often proven elusive to detect experimentally (Lessells, 1985; Lyons, 1962; Walde & Murdoch, 1988). Studies of parasitoid responses to EAB densities are limited, and we do not know if these introduced parasitoids will aggregate or attack hosts in a density dependent manner. One study found no evidence of a positive relationship between parasitism and larval density in green ash trees, *F. pennsylvanica*, that had high densities of EAB, but did find evidence of a positive relationship between parasitism and EAB larvae densities in oriental ash, *F. rhynchophylla*, with moderate/low densities of EAB (Duan & Oppel, 2012).

There is evidence, however, that woodpeckers cause density-dependent mortality to EAB (Flower et al., 2014; Koenig et al., 2013). Many studies have found that woodpecker predation is positively associated with increasing EAB density and these studies support both a numerical response (more woodpeckers) and a Type III functional response (shift in foraging towards ash, (Holling, 1965)) of woodpeckers to EAB densities at a stand-level(Flower et al., 2014; Koenig et al., 2013). The latter response would be expected to increase the rate of woodpecker predation as a function of EAB density up to some density threshold, above which the predation rate would decline with density, because the woodpeckers are satiated. This experiment showed precisely this increase in predation rates on control trees (Fig. 5), but I did not see the same response on trees with parasitoids. Thus, it is possible that the presence of parasitoid influenced the shift in woodpecker behavior responsible for a Type III functional response. It is important to note that other studies have found that a relationship between woodpecker predation and EAB density is not always evident (Duan et al., 2010; Flower et al., 2014).Koenig et al. (2013) found mixed results, while red-bellied woodpeckers, and white-breasted nuthatches, *Sitta canadensis,* experienced numerical increases and downy and hairy woodpecker populations had numerical decreases as EAB infestations progressed from moderate to high densities. These results demonstrate that while predation is positively correlated with larval density among the control trees, this correlation disappears, or in the case of *T. planipennisi* treatment trees, reverses when woodpeckers feed on treatment trees with parasitized larvae (Fig. 5; Table 4). This change in behavior is consistent with my low-food reward theory discussed above.

Discussion of density dependence is complicated by the fact that the nature of a density-dependent relationship between natural enemies and their hosts may reverse or disappear as host density changes. Analyses of the gypsy moth (*Lymantria dispar* [L.]) system, have suggested that while predators may stabilize low density populations through positively density dependence mortality, they do not control outbreaking populations (Campbell, 1975; Campbell & Sloan, 1977). Applied to the EAB system, interactions between predators and parasitoids may change over time as EAB populations rise, fall, and crash, as most ash trees in an EAB- infested stands die.

If both parasitoids and predators seek out the same trees where EAB is at high density, then the interaction will be complicated and not necessarily beneficial for EAB population control. However, if they seek out different trees their interactions are more likely to be synergistic. In Michigan, Duan et al. (2013) found no difference between sites with or without the establishment of *T. planipennisi*. However, Duan et al. (2013) compared predation rates overall and not tree by tree, so it is possible that woodpeckers were still avoiding trees with a higher percentage of parasitized EAB within the stand. Only further studies in stands with and without established parasitoids could determine whether either of these explanations might be true.

In conclusion, I found strong evidence that woodpeckers do not discriminate between parasitized and non-parasitized larvae at the tree-level. However, at the stand-level, I found evidence of significant decrease in woodpecker predation when woodpeckers encounter moderate to high parasitism rates. I believe this is because parasitism contributes to reduced patch quality and when encountered by woodpeckers they choose to quit foraging sooner than if they encounter the same density of non-parasitized larvae. Implications for biological control and management of EAB are hard to gauge from out study alone, as the effects could be positive if woodpeckers are foraging in a way that reduces predation of parasitized larvae. However, the impact could be negative if the presence of parasitized larvae is great enough to reduce the overall quality of food available in ash trees and cause woodpecker foraging to shift away from ash. More work needs to be done to further recognize the implications of the intriguing and important interaction between woodpecker predators and parasitoids of the emerald ash borer.

CHAPTER 2

CAN *SPATHIUS GALINAE* ATTACK EMERALD ASH BORER LARVAE FEEDING IN LARGE ASH TREES?

2.1 Introduction

Tetrastichus planipennisi is the most widely established introduced biological control parasitoid of the emerald ash borer in North America (Bauer et al., 2015), however, a previous study by Abell et al. (2012) found that this parasitoid species cannot oviposit in the lower boles of trees with a bark thickness exceeding 3.2 mm (equal to trees with a DBH > 11.2 cm) due to its short ovipositor. This creates a large refuge for EAB larvae, particularly in stands with more mature ash trees. Data from the USFS shows that as of 2014, over 500 million ash trees or 26% of all *Fraxinus* spp., on forested land in the Northeast[2] were too large for *T. planipennisi* to successfully oviposit in at breast height (FIDO 2009-2014). *Tetrastichus planipennisi* parasitism can still occur, however, on larvae in the upper bole and smaller branches of these larger trees (Duan pers. comm.). *Spathius agrili* was also approved for release in 2007 and has a longer ovipositor than *T. planipennisi,* but this species has failed to establish north of the 40th parallel (USDA–APHIS/ARS/FS., 2016). This means that many EAB larvae in large ash trees remain inaccessible to introduced larval parasitoids, highlighting the need for another, introduced parasitoid with a longer ovipositor able to target the larvae in these larger trees.

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[2] Connecticut, Maine, Massachusetts, New Jersey, New York, Pennsylvania, Rhode Island, Vermont.

Spathius galinae, which was approved by the USDA for release in 2015, has a longer ovipositor than *T. planipennisi.* The ovipositor of *S. galinae* is 4 to 5.3 mm in length, while that of *T. planipennisi* is only 2.0 to 2.5 mm (Duan & Oppel, 2012; Gould & Duan, 2013). On the basis of morphology, *S. galinae* should be able to attack hosts in larger ash trees (Gould & Duan, 2013). *Spathius galinae* is promising as an additional control of EAB because climate matching suggests that there is a better fit between its native range and that of north central and northeastern U.S. than is true for *S. agrili* (Duan & Oppel, 2012; Gould & Duan, 2013). In addition to its long ovipositor, *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). My study aims to further quantify the potential success of *S. galinae* by investigating how oviposition success *changes* as bark thickness increases.

The effect of bark thickness on parasitism has previously been tested using both *T. planipennisi* and *S. galinae* by (Wang et al., 2015). In their study, they found that parasitism rates of *T. planipennisi* on large logs were significantly lower than on small logs (Wang et al., 2015). They found no significant difference in parasitism rates for *S. galinae* across log sizes (Wang et al., 2015). However, in their experiment the large logs did not exceed 10 cm diameter. While this diameter is close to the upper limit (11.2 cm) for *T. planipennisi,* it is hypothesized 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, I examined conditions closer to *S. galinae'*s expected oviposition limit and examined whether a similar change in parasitism is noticeable for *S. galinae* as it approaches its oviposition limit. Knowing *S. galinae's* oviposition limits would improve EAB management through a greater understanding of the expected impact of *S. galinae* under field conditions and would assist in any 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 to eliminate larvae in trees inaccessible to larval parasitoids, such as using trunk injections of pesticides or selected tree removal.

2.2 Methods

2.2.1 ***Overview:*** Under controlled conditions in the laboratory, white ash (*Fraxinus americana*) logs were artificially infested with EAB larvae and then introduced to parasitoids in cages after larvae had reached the 3rd or 4th instars, a suitable age for parasitism (Duan et al., 2013; Duan et al., 2014). After exposure, potentially parasitized larvae were allowed to develop for two weeks and then all logs were peeled to record the fate of the EAB larvae (alive, dead, parasitized) and parasitism rates were calculated (Duan et al., 2010).

2.2.2 ***Log selection:*** White ash logs of different diameters (small= 3-8, medium = 12-18, large = 25-30 cm) were cut 10 to 30 cm in length depending on diameter to keep bark area the same among treatments. Logs lengths and number of logs varied among treatments (four to eight small logs, two to four medium logs, and one to two large logs) such that in aggregate the logs had the same bark surface area available to *S. galinae*.

2.2.3 ***Egg application:*** To inoculate logs with EAB, 20 eggs were applied to each treatment (log or group of logs). EAB eggs were provided by the USDA-APHIS, EAB Rearing Facility (Brighton, MI, USA) 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 glued to the bark, with the eggs facing out, padded with a cotton ball, and secured to the log with breathable quick-dry ribbon. This technique was modified from Abell et al. (2012) to accommodate the large logs that were used in this study. Before egg application, any rough bark surfaces were lightly scraped to create a flat, smooth surface for egg placement. These steps ensured the eggs lay flush on the bark, so that the neonate larvae could successfully access the log upon hatching. Logs were then placed with the lower cut end sitting in plastic trays with 2 cm of water in a climate-controlled room or chamber with RH >60%. After two to three weeks, the filter paper squares were removed and the number of hatched eggs counted. Initial experiments were conducted in a climate-controlled room held at 30°C during larval development and 25°C during parasitoid exposure. When I switched to climate-controlled chambers both larval development and parasitoid exposures were conducted at 25°C. Lighting was diffuse, with a 18:6 L:D cycle. The water was changed bi-weekly and the cut ends of the logs were scrubbed to prevent excessive growth of mold or algae. After approximately 4 to 6 weeks, when EAB larvae had developed to $3^{rd}/4^{th}$ instars, logs were exposed to parasitoids.

2.2.4 *Parasitoid exposure:* Logs were grouped to ensure consistent surface area and approximately the same number of larvae per treatment, based on hatch records. Once grouped, logs were placed in mesh cages, large logs in 45 cm^3 cages, medium and small logs in 30 cm^3 cages, all with 680 µm polyester mesh (BioQuip, Rancho Dominguez, CA), during the initial two trials and in similar cages, 27 cm^3 with 24 x 24 mesh plastic screen (BioQuip, Rancho Dominguez, CA) during the last trial conducted in the climate-controlled chamber. Logs were exposed to *S. galinae* females in approximately a 2:1 wasp: host ratio based on egg hatch rates. Logs with mature EAB larvae were exposed to one-week to two-week old mated, naïve *S. galinae* for two weeks to provide opportunities for parasitism under the same conditions as describe above for larval rearing. Logs were then removed from cages and returned to plastic trays with water for an additional two weeks to allow parasitoids to develop under the same temperature and light conditions as above.

2.2.5 ***Detection of parasitism:*** To determine the percentage of EAB larvae successfully parasitized in logs of different diameters, logs were debarked with a draw knife or chisel to locate larvae and determine their parasitism status. Three to five random, intact vertical bark segments were kept from each log, to find bark thickness as discussed below.

2.2.6. ***Measuring bark thickness in experimental logs.*** To determine the thickness of the bark in the experimental logs, all bark segments were cut across the grain with a band saw and the cut edge was sanded with an electric sander to delineate between the inner and outer bark. Unlike the Abell et al. (2012) study, my measurements incorporated both outer and inner bark (phloem), because larvae were found feeding at the intersection of the inner bark and cambium (Poland & McCullough, 2006). Thicknesses of valleys (furrows) and ridges of inner and outer bark were recorded, averaging two thickness measurements per piece of bark with digital calipers. Only valley thickness were used in these analyses (Abell et al., 2012). Whenever it was possible to get a complete bark sample from above a parasitized or non-parasitized larva, I measured valley bark thickness directly above the larva and bark thickness in the valley immediately adjacent to the larva.

2.2.7 ***Trial Summary*** Three trials of the experiment were run between July 2015 and December 2015. Logs were cut within 3 days of starting each trial. Trial 1 had an egg incubation period of 4 weeks, 28 July 2015-27 August 2015, and exposure to parasitoids period of 16 days, 27 August 2015-12 September 2015. Trial 2 had an egg incubation period of 6 weeks, 08 September 2015-21 October 2015, and exposure period of 14 days, 21 October 2015-4 November 2015. Trial 3 had an egg incubation period of 6 weeks, 07 October 2015-23 November 15, and exposure period of 14 days 23 November 2015-7 December 2015.

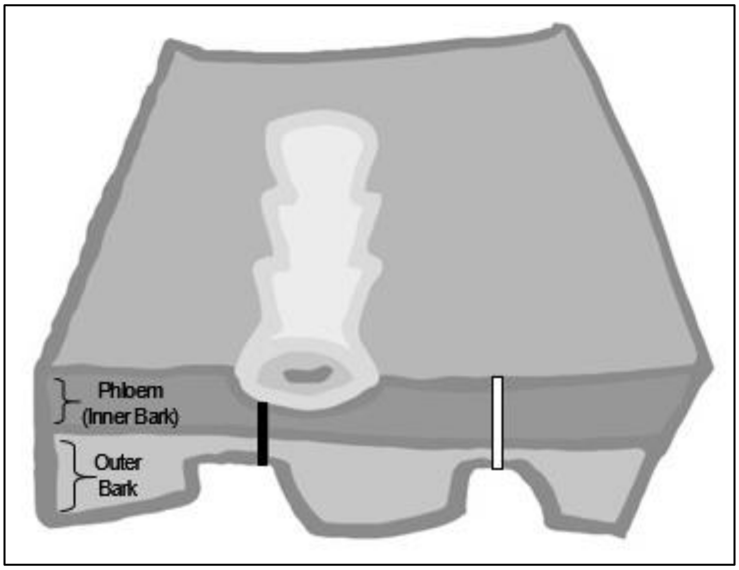

Figure 7 A diagram of bark measurements: **White-** valley thickness **Black-** valley thickness over the parasitized larvae.

2.2.8 ***Measuring bark thickness in the field:*** To help understand how tree size in the field relates to bark thickness, bark samples were taken from ten white ash trees of various larger diameters at two sites in Pittsfield, Massachusetts and Hamden, Connecticut. Bark thickness samples and diameter measurements were taken from each tree at two heights, 0.5 m and 1.4 meters. Tree diameter was recorded at each height and then a leather punch (2.5 cm dia) and mallet were used to collect three bark plug samples from each tree, one each from the S, NW and NE. Average valley bark thickness was measured for each bark sample by taking two valley measurements per sample and averaging together all six valleys measurements per diameter.

Additional field data (from several sites throughout New York) was provided by USDA-APHIS-PPQ in which average bark thickness per one meter tree segment was found by taking valley measurements at three, equally-spaced, randomly selected points along the perimeter of the tree segment. Diameter of each tree segment was also provided in the data set. These data were incorporated into my analysis of average bark valley thickness versus diameter.

2.2.9 ***Statistical Methods:*** Logistic regression was used to evaluate the effect of bark thickness on the probability of parasitism. I used a generalized linear mixed-effects model (GLMM) with binomial error distribution and logit link. Proportion of parasitized larvae served as the response variable and bark thickness and exposed surface area as predictors. To help with model convergence, within each trial the surface area was standardized by dividing surface area by the average surface area across all cages. Although I accounted for surface area in my original model, I dropped it in the final model because it was not significant and dropping it improved the explanatory power of the model using Akaike's Information Criterion (AIC). To account for small differences in exposure length and cage type between trials, trial and cage type nested within trial were incorporated as random effects.

Another GLMM was used to test for differences in standard valley bark thickness over-galleries to valley thicknesses not-over-galleries, to determine how larval feeding on phloem tissue reduced the thickness of the bark immediately above the gallery. To account for overdispersion observed in the data I used a gamma error distribution and inverse link. Log identity was incorporated as a random effect to account for variability in bark thicknesses between logs.

Lastly, the relationship of average bark thickness to diameter was analyzed with both a linear and logarithmic lines of best fit, data from all laboratory, field, and USDA experiments were combined into one dataset before analysis. All analyses were conducted in R version 3.2.5 (R Core Team, 2013).

2.3 Results

2.3.1 ***Effect of bark thickness on parasitism.*** Percentage parasitism declined significantly with increasing valley bark thickness (Table 5 & Fig. 8: DF=1, P= .004). When bark thickness was recorded over galleries with parasitized larvae, parasitism was found in ash logs with average bark valley thickness up to 4 to 5 mm (Fig. 9). A boxplot of minimum thicknesses measured directly above galleries of parasitized larvae ranged from 0.95 mm to 5.5 mm, with an average of 2.7 mm. (Fig. 9A).

2.3.2 ***Comparison of bark thickness above vs not above EAB galleries.*** Bark in valleys directly above EAB galleries was significantly thinner than bark not over EAB galleries, by an average difference of 0.41 mm (*P* <0.001, GLMM) (Table 6; Fig. 10). For galleries above EAB larvae, both larvae parasitized by *S. galinae* and non-parasitized larvae were grouped prior to analysis, because previous statistical analysis confirmed that the bark thicknesses above *S. galinae*-parasitized EAB larval galleries did not differ statistically from thicknesses above the galleries of non-parasitized larvae. Also, bark thickness above larvae differed significantly from bark thickness of a valley directly adjacent to that larvae, by an average of -0.41 mm as determined by a non-parametric lower-tailed Wilcoxon signed-rank test (*V*=283.5, *P*<0.0001).

2.3.3 ***Relation between bark thickness and tree size in the field.*** Average valley bark thickness was compared to log diameter (DBH) with both a linear ($R^2 = 0.55$) and logarithmic ($R^2 = 0.57$) model. The best logarithimic model, y=1.898*ln(x)-1.064, where y is bark thickness and x is diameter, shows that bark thickness levels off at 6 to 8 mm for trees up to 83 cm DBH (Fig. 12).

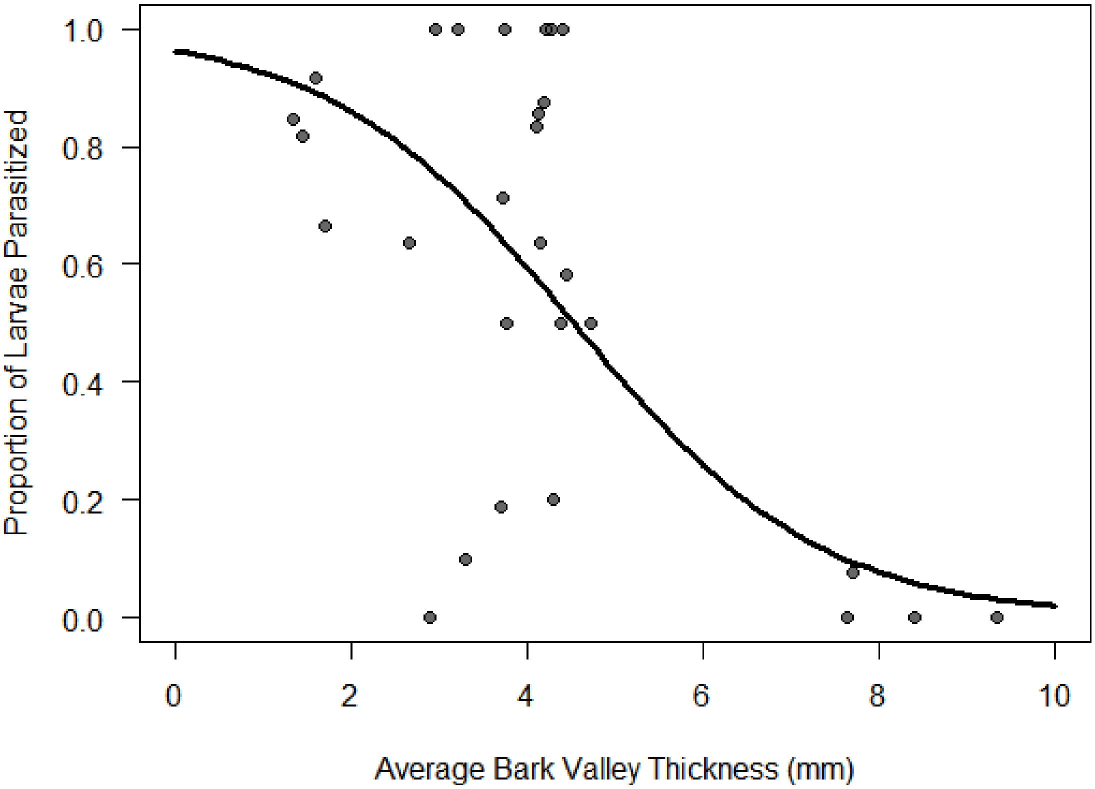

Figure 8 A fitted logistic regression of the proportion of larvae parasitized from the laboratory experiments as a function of average bark valley thickness using the visreg function in R. (See Table 5 for model details)

Table 5 Parameter estimates and statistical significance of various fixed and random effects of proportion of larvae parasitized by *Spathius galinae* weighted by total larvae. This model is a logistic regression with binomial error distribution and logit link.

Fixed Effects	**Estimate**	**SE**	**z-value**	***P***
(Intercept)	3.243	1.200	2.705	0.00689**
Average Valley Thickness	-0.716	0.255	-2.808	0.00499**
Random Effects		**Variance**	**Std. Dev.**	
Cage:Trial	(Intercept)	1.8679	1.3667	
Trial	(Intercept)	0.4717	0.6868	

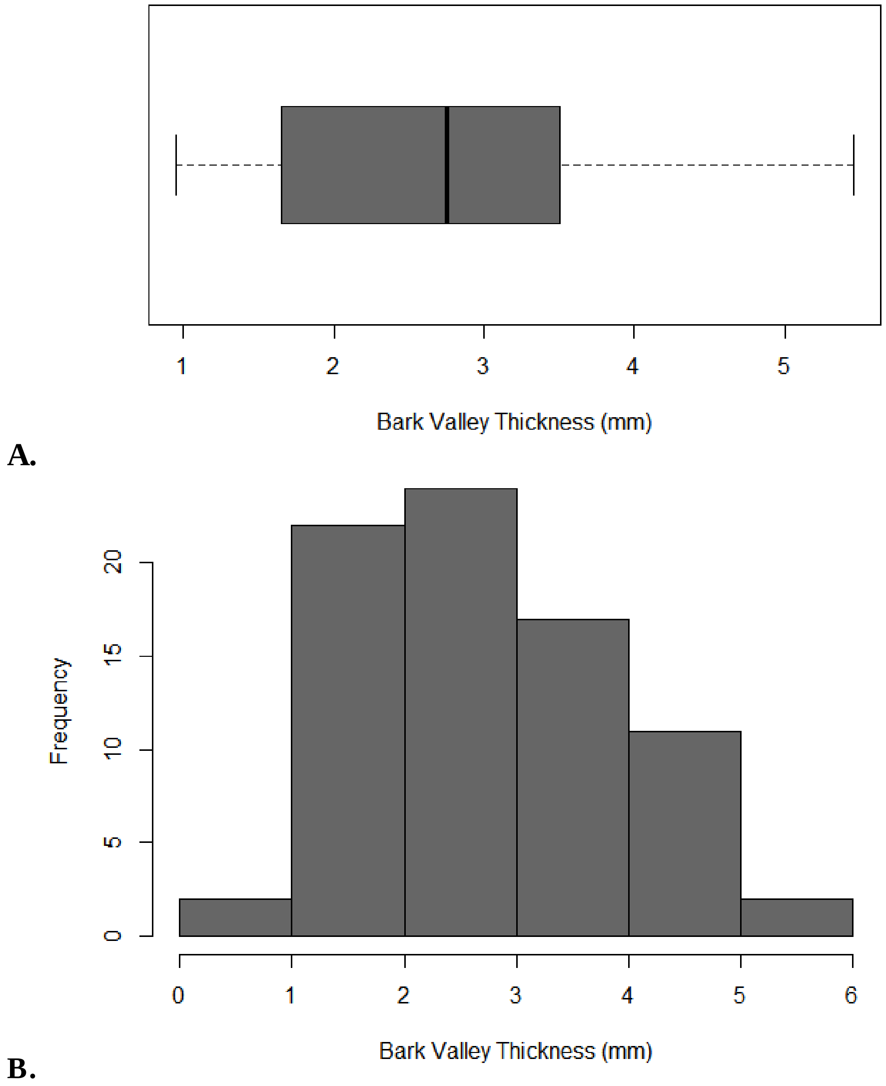

Figure 9 (A) boxplot and (B) histogram illustrating the distribution of valley thicknesses above all EAB, *Agrilus planipennis*, larvae from the laboratory experiment that were parasitized by *Spathius galinae*.

Figure 10 Effect of galleries on reduction in valley bark thickness from the laboratory experiment. The black line is for standard bark valley thickness and the grey line is for bark valley thickness over EAB, *Agrilus planipennis*, galleries. Valley thickness over gallery was significantly less than standard valley thickness. (See Table 6 for model details)

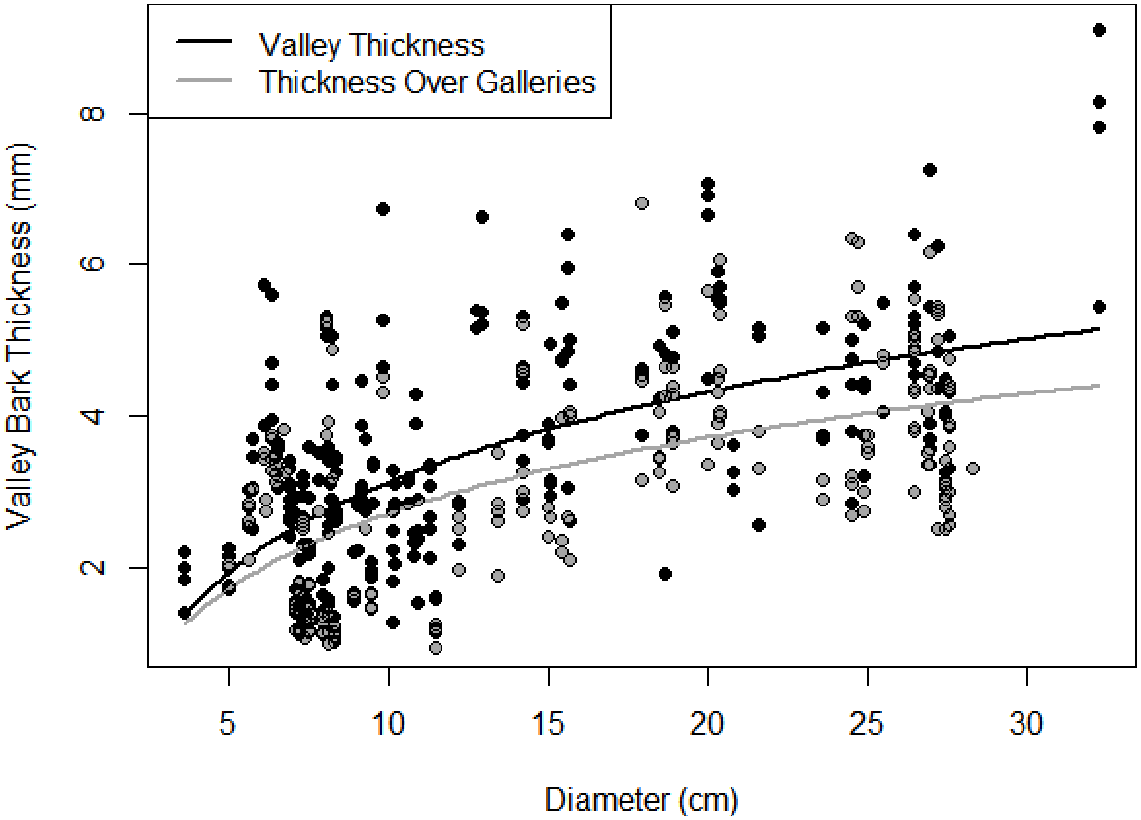

Table 6 Parameter estimates and statistical significance of various fixed and random effects of bark valley thickness (mm), determined by generalized linear mixed model (GLMM) with gamma error distribution and inverse link.

Fixed Effects	**Estimate**	**SE**	**t-value**	***P***
(Intercept)	0.65423	0.093339	7.009	2.40E-12
Natural Log of the Diameter	-0.10916	0.039497	-2.764	0.00571
Thickness Over Galleries	0.037427	0.005497	6.808	9.87E-12
Random Effects		**Variance**	**Std. Dev.**	
Log ID	(Intercept)	0.008574	0.0926	
Residual		0.047092	0.2170	

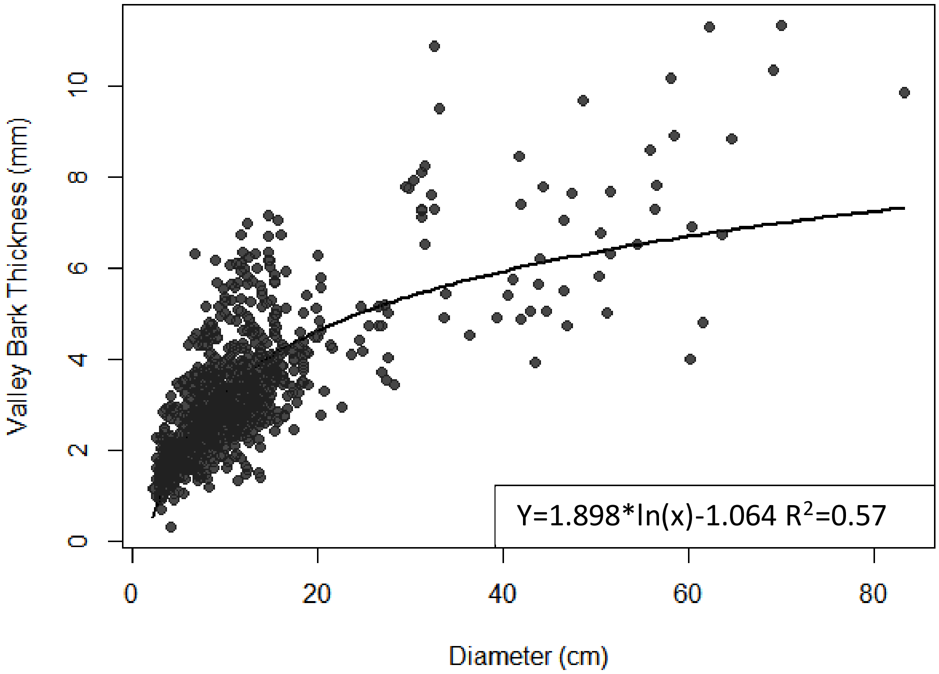

Figure 11 A logarithmic regression comparing average valley bark thicknesses of a log (mm) to the natural log of the diameter of the log (cm) for white ash, *Fraxinus americana*, from combined laboratory, field and USDA datasets.

2.4 Discussion

Our results suggest that the limit to *S. galinae* oviposition success lies between 4 and 8 mm (Fig. 8). Based on the measurements of minimum valley thicknesses recorded directly above galleries of parasitized larvae, this limit is estimated to be around 5.5 mm (Fig. 9). Because *S. galinae* paralyzes the larvae during oviposition, I know that it must have accessed the bark directly over the end of the larvae gallery. Taking the minimum valley measurement above that gallery thus gives us a conservative estimate of bark thickness through which it is capable of ovipositing (Watt & Duan, 2014). This potential limit of 5.5 mm matches closely with the average length of *S. galinae*'s ovipositor of 4 to 5.3 mm (Gould & Duan, 2013).

In addition I found a significant difference between average measurements and measurements directly above galleries (Fig. 10), which demonstrates that *S. galinae* only needs to access approximately 0.4 mm smaller depth of bark, to parasitize a larva than suggested by measurements of average valley thickness of a given log. Thus if *S. galinae's* limit were found to be around 5.5 mm this would be equivalent to an average valley thickness of 5.9 mm.

Our regression estimate of a bark thickness limit of 5.9 mm corresponds to a 39.2 cm diameter (Fig. 12). This finding suggests that *S. galinae* would be able to exceed the bole limit of *T. planipennisi* (11-12 cm DBH) by about four times. Due to the nature of a plateauing logarithmic function a small increase beyond 5.9 mm would see a marked increase in diameter limit. If the limit of *S. galinae* is moderately beyond my conservative limit of 5.9 mm, which is not unreasonable given the unfortunate gap in the bark thicknesses I was able to test in the medium thickness range of 12 to 18 cm, due to a consistent but currently inexplicable failure of larval development in these logs. A small increase of bark limit, to 6.5 mm results in a large increase in diameter limit to 53 cm, close to five times the limit of *T. planipennisi.*

Using my most conservative estimate of a limit (<5.9 mm in valley bark thickness, <39.2 cm DBH) and data from the USFS for Massachusetts forests[3], *S. galinae* would be able to attack EAB larvae at the DBH of 94% of *Fraxinus* spp. trees (FIDO 2009-2014).

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[3] Measurements are done using estimates of tree counts and discount any saplings under 2.5 cm diameter.

By my more liberal estimate of limit (<6.5 mm in valley bark thickness, <53 cm DBH), EAB larvae would be accessible to attack by *S. galinae* in 98.7% of the ash trees in Massachusetts (FIDO 2009-2014). Expanding these estimates to the northeastern region[4] only 2.5% of forest ash in the region are larger than 39.2 cm DBH (>5.9 mm) and only 0.4% are larger than 53 cm DBH (>6.5 mm) (FIDO 2008-2012).

Spathius galinae thus seems to be highly suitable to fit into a biocontrol program providing protection for ash through fairly large sizes (39-43 cm DBH). When viewed as a series of life stages, the life of an ash tree can be divided into sapling (< 5 cm DBH), pole size trees (5-12 cm), and mature trees of moderate to large size (15-50 cm). For saplings, *T. planipennisi* currently in Michigan parasitizes 40-70% of susceptible larval stages (3rd-JL) present (J. Duan and R. Van Driesche, unpub. data). For pole size ash trees (7-15 cm DBH) in Michigan, *T. planipennisi* attacks up to 22 % of suitably sized larvae (Duan et al. 2013), with a likely upper limit of 11 cm (for the lower bole) (Abell et al. 2012). The remaining ash tree life stage of mid-sized to large trees will be the category for which *S. galinae* is adapted. Many managers have suggested that the largest role for these introduced biocontrol agents may be in the aftermath of an EAB invasion, in maintaining EAB populations at low levels to permit survival and regeneration of ash (Bauer et al. 2015). For ash trees in aftermath areas, establishing a complex of biological control agents is likely the only management strategy that will allow ash to renew itself on the landscape (Duan et al. 2013). *Spathius galinae* may thus prove vital in allowing ash to reach larger sizes as they age and mature in recovering forests.

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[4] Connecticut, Maine, Massachusetts, New Jersey, New York, Pennsylvania, Rhode Island, Vermont.

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