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Establishment of classical biological control targeting emerald ash borer is facilitated by use of insecticides, with little effect on native arthropod communities

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12 Abstract

13 Emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), is rapidly invading North
14 America and has inflicted extensive ash, *Fraxinus* spp., mortality in affected areas, altering
15 composition and structure of wildland and urban forests. Insecticides can effectively protect ash
16 on a small scale and classical biological control efforts have been implemented. In the U.S.
17 emerald ash borer kills native ash so quickly, and populations are so mobile, that establishing an
18 effective biological control program is challenging. Consequently, we assessed the compatibility
19 of using reduced rates of the insecticide imidacloprid applied as a soil drench coupled with
20 releases of three species of parasitic wasps. In this way we hope to slow the process of ash
21 decline and prolong the window of opportunity to allow biological control agents to become
22 established. We compared the efficacy and effects of i.) full strength imidacloprid applications,
23 ii.) reduced rates of imidacloprid coupled with classical biological control, iii.) classical
24 biological control alone, and iv.) untreated controls. We monitored emerald ash borer adult
25 activity through trapping and destructive sampling for larvae, evaluated larval parasitization
26 rates, and also assessed the native hymenopteran communities. Chemical treatments were not
27 adequate to prevent ash canopy decline, but they also did not impede parasitization of emerald
28 ash borer eggs or larvae. Two of the three classical biological control agents, *Oobius agrili* and
29 *Tetrastichus planipennisi*, were recovered from trees treated with imidacloprid, demonstrating
30 that they were successfully reproducing and indicating the compatibility of the two management
31 strategies. Non-target hymenopterans appeared unaffected by the insecticide applications.
32 Importantly we found no negative effects of the imidacloprid applications on native pollinators.
33 Collectively these data suggest that judicious use of insecticides coupled with classical biological

34 control may be an effective and potentially sustainable approach to managing invading
35 populations of emerald ash borer.

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37 Keywords: *Agrilus planipennis*, parasitoids, imidacloprid, sustainable, invasive, non-targets,
38 pollinators

1. Introduction

Ash, *Fraxinus* spp., are a consistent component of hardwood forests throughout the United States (Schlesinger, 1990) and comprise a significant component of urban trees in many cities (Cappaert et al., 2005). In the U.S. the prevalence of ash is threatened by the emerald ash borer, *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an invasive beetle native to eastern Asia that was first detected in the United States in 2002 (Haack et al., 2002). Emerald ash borer is a wood-boring, phloem-feeding insect that destroys the vascular system of infested trees, causing rapid tree mortality. Since its introduction emerald ash borer has spread throughout much of the contiguous U.S. and southeastern Canada (www.emeraldashborer.info/files/MultiState_EABpos.pdf), inflicting extensive ash mortality throughout affected regions. With the notable exception of blue ash, *F. quadrimaculata*, which demonstrates some putative resistance (Tanis and McCullough, 2015; Peterson et al., 2015), all North American *Fraxinus* are susceptible to attack (Rebek et al., 2008; Tanis and McCullough, 2015), and emerald ash borer readily colonizes healthy trees (Cappaert et al., 2005).

In its native range, emerald ash borer populations are regulated by resistance of Asian *Fraxinus* species (Rebek et al., 2008) and a diverse assemblage of natural enemies capable of inflicting high rates of mortality (Liu et al., 2003; Zhang et al., 2005; Duan et al., 2012b; Wang et al., 2016). The lack of co-evolved tree defenses and natural enemies contribute to the invasiveness of emerald ash borer in North America. Large trees can be killed in as little as three years following initial infestation and complete ash mortality in an area can occur in as little as five years (Poland and McCullough, 2006).

Chemical insecticides can be used to protect trees from attack (Smitley et al., 2010a; Smitley et al., 2010b; Herms et al., 2014; McCullough, 2015; Smitley et al., 2015), but many of

the more effective approaches require the use of specialized equipment by certified applicators (Herms et al., 2014). Imidacloprid can be applied as a soil drench, but as a neonicotinoid it has come under increased scrutiny as a potential contributor to pollinator decline (Decourtye and Devillers, 2010; Whitehorn et al., 2012; Goulson, 2013). Chemical controls effectively cause emerald ash borer mortality but also inflict high rates of mortality on natural enemies. Furthermore, chemical suppression is economically and ecologically unsustainable over large areas and for extended time periods. Consequently, considerable effort has been spent developing a classical biological control program against emerald ash borer, the effectiveness and effects of which are not fully understood (but see Duan et al., 2014; 2015). Several hymenopteran parasitoid species have been imported from eastern Asia and are being reared in the U.S. for deployment throughout infested regions. Two of these, *Spathius agrili* Yang (Braconidae) and *Tetrastichus planipennisi* Yang (Eulophidae), are larval parasitoids (Yang et al., 2005; Yang et al., 2006) while the third, *Oobius agrili* Zhang (Encyrtidae) (Zhang et al., 2005), is an egg parasitoid. Releases have been ongoing since 2007 (USDA APHIS, 2007) and parasitoids have been routinely deployed throughout the invaded range (<http://www.mapbiocontrol.org/>). *Tetrastichus planipennisi* and *O. agrili* have readily established populations in release locations and their populations are increasing, although they have been unable to reach numbers rapidly enough to sufficiently suppress emerald ash borer populations and prevent ash mortality (Abell et al., 2014; Duan et al., 2013; 2014; 2015).

Due to the rapid rate at which emerald ash borer kills trees and the time required for classical biological control agents to attain sufficient numbers for population suppression, biological control alone has not been adequate to prevent widespread ash mortality in North America. Blending the two approaches, utilizing insecticide applications to slow ash decline

while simultaneously mass-releasing parasitoids, thereby creating a longer window of opportunity for parasitoids to establish and reproduce (Katovich and McCullough 2010), appears plausible and may be more sustainably effective than either approach alone. However, the compatibility of these suppression methods has not been fully evaluated (McCullough, 2015). We evaluated the feasibility of employing this potentially sustainable approach to emerald ash borer management by blending biological control releases with reduced rates of chemical insecticides. Compatibility of the two mitigation strategies was investigated along with their impact on emerald ash borer populations, ash decline, and non-target native insects, including pollinators.

2. Methods and materials

2.1 Study Sites

Sites were established in north-central Kentucky along the forefront of the expanding emerald ash borer infestation in a region characterized by a Western Mesophytic forest type consisting of a wide variety of deciduous species (Wharton and Barbour, 1973) and with a significant ash component (Davidson and Rieske, 2015). Sites (one per county) were selected in Anderson, Fayette, Henry, Shelby, and Spencer counties, where emerald borer has been reported since 2009-2010. In Anderson, Henry, and Shelby emerald ash borer was present at the onset of the study and forests showed varying levels of ash decline, typified by crown thinning, branch dieback, splitting bark, and woodpecker activity, whereas at Fayette and Spencer sites the first detection was made in 2014 and there were little to no signs of ash stress.

2.2. Plot treatments

Each site consisted of three blocks of four 0.04 ha plots, separated by > 100 m, with each block containing one replicate of each of four treatments: 1) insecticide application (“chem”), 2) biological control releases (“bio”), 3) a dual treatment coupling reduced rate insecticide applications with biological control releases (“dual”), and 4) untreated controls. Thus, each site contained 12 plots (n = 60). In plots designated for insecticide application, all ash stems > 5 cm at 1.5 m above ground level (DBH) were treated with imidacloprid (Xytect 75WSP, RainbowTreecare, Minneapolis, MN) applied via soil drench within 0.5 m of tree stems. The full strength imidacloprid treatment was generated by diluting 1.48 g product/ cm DBH in 3.79 liter of water; this was equivalent to 1.12 g ai/ cm DBH (Smitley et al., 2015). In dual treatment plots where reduced chemical rates were combined with biological control releases, imidacloprid was applied at half of the recommended dose (i.e., 0.56 g ai/ cm DBH). Initial chemical applications were made between Mar. 08 and Mar. 27 2013 and were reapplied between Mar. 14 and Mar. 21 2014. Since soil moisture affects imidacloprid uptake (Smitley et al. 2015) we also noted precipitation in the time period 7 d prior and 21 d following applications and calculated the deviation from normal for the same time period (Table 1). In the dual treatment and biological control plots 18,698 *T. planipennisi* were deployed equally across plots at Anderson, Henry and Shelby between Aug. 30 and Oct. 28 2013 (Table 1). Releases were not made at Spencer or Fayette as emerald ash borer had yet to be detected at those sites. In 2014 85,200 *T. planipennisi* were deployed in addition to 10,500 *S. agrili* and 19,650 *O. agrili*; these deployments were carried out weekly from May 01 through Sep. 30 (Table 1). First detection of emerald ash borer at Fayette occurred May 28 2014 and weekly parasitoid deployment began on Jul. 15. The majority (~80%) of *T. planipennisi* and *S. agrili* were deployed as larvae within ~20 cm × 10 cm

ash bolts containing parasitized emerald ash borer larvae; the remainder were released as adults. *Oobius agrili* were deployed as larvae within parasitized emerald ash borer eggs on filter paper and contained within 0.5 liter plastic cups. The cups had mesh screen over the opening and were inverted and fastened to ash trunks within designated plots (USDA APHIS, 2013). All parasitoids were obtained from the USDA APHIS PPQ Parasitoid Rearing Facility in Brighton, MI.

2.3 Forest characteristics

Whole (0.04 ha) plots were utilized to assess overstory and midstory vegetation (all trees ≥ 12.7 cm diameter at 1.5 m high; DBH), 0.004 ha sub-plots were used to assess saplings and shrubs (< 12.7 cm DBH, > 137 cm high), and 0.0004 ha micro-plots were used to assess seedlings and shrubs (< 137 cm height). One sub-plot and one micro-plot were positioned at the whole-plot center and in each cardinal direction 7.7 m from the whole-plot center. Thus, a surveyed plot contained one 0.04 ha whole-plot, five 0.004 ha sub-plots, and five 0.0004 micro-plots (Coleman et al., 2008). Measurements of vegetation and plot data followed the Common Stand Exam protocol of the USDA Forest Service's Natural Resource Information System: Field Sampled Vegetation Module (USDA Forest Service, 2003) and included tree height and DBH. Canopy dieback was visually assessed by a single observer in summer 2014, and each ash tree assigned a crown dieback rating from 0% (healthy) to 100% (dead) (Davidson and Rieske, 2015).

2.4 Emerald ash borer intercept traps

Emerald ash borer activity was monitored using green Lindgren 12-unit funnel traps fitted with manuka oil and leaf alcohol lures (Synergy Semiochemical, BC). Traps (1 per plot) were deployed May 05 to Jul. 17 2014 by suspending over the highest reachable ash branch (~5-10 m). Trap contents were collected weekly and returned to the laboratory where adult emerald ash borer were counted.

2.5 Destructive Sampling

Twenty-five total ash ranging in size from 6.1-15.2 cm DBH (mean 8.8 ± 0.29 cm) were felled in February and March of 2014 across all sites where emerald ash borer was evident (Anderson, Henry, and Shelby) and an additional 15 total were felled in March 2015. Over both years nine trees were felled from plots receiving full strength imidacloprid treatments (chem.), ten each from dual treatment (dual) and biological control (bio) plots, and 11 from untreated control plots (control). Felled trees were cut into 60 cm sections which were numbered sequentially from the base and returned to the laboratory for immediate de-barking and for parasitoid rearing. Odd numbered log sections were debarked using a draw knife and emerald ash borer larvae counted and inspected for signs of parasitism. Immature parasitoids were counted, placed with moistened filter paper inside 100 mm diameter petri dishes sealed with Parafilm, and reared to adulthood for identification. Length and mid-section diameter of debarked logs were recorded.

To assess *O. agrilus* establishment sampling was conducted in 2015 only, when two logs from each tree felled ($n = 15$) were evaluated. Bark was shaved with a draw knife from the apical 300 cm² of logs at trunk heights of 0.6 m (corresponding to log section 1), and 5.5 m (corresponding to log section 9). Shavings were sifted through a 1 mm sieve and sorted under 6 –

40× magnification using a dissecting microscope to separate healthy golden-colored emerald ash borer eggs from darker colored parasitized eggs (Abell et al., 2014). Even numbered log sections were placed in cylindrical mesh rearing enclosures (30 cm × 80 cm) fitted with 7 mL clear collection vials. Rearing enclosures were checked daily for four months for emerging parasitoids. Logs were removed from enclosures following cessation of insect emergence and debarked as described previously to check for unemerged parasitoids.

2.6. *Native insect communities*

During the summers of 2013 and 2014 native insects were monitored with pan traps which consisted of two nested yellow plastic bowls (16 cm × 4 cm, Festive Occasion, East Providence, RI), with the internal bowl secured with binder clips to the outer bowl and filled with 75 mL soapy water. Traps were affixed to 3 cm × 3 cm wooden stakes 75 cm above ground level and 5 m north of the plot center. Insects were collected from pan traps by straining contents through paper filters (Rockline Industries, Sheboygan, WI), placing in sealable plastic bags with 70% EtOH, and storing at 4 °C until processing. In the laboratory insects were sorted to order (Triplehorn and Johnson, 2005) and counted. Hymenopterans collected in 2014 were further divided into Apoidea (sans Sphecidae), Formicidae, Ichneumonoidea (Ichneumonidae and Braconidae), non-ichneumonoid parasitoids (superfamilies: Ceraphronoidea, Chalcidoidea, Chrysidoidea, Cynipoidea, Diaproidea, and Platygastroidea) (Gibson et al. 1997), Symphyta, and Vespidae.

Concurrent with pan trapping, Malaise traps (2.44 m² × 2.13 m high, 1.89 L collection head, BioQuip, Rancho Dominguez, CA) were deployed 5 m south of each plot center with a 2

cm × 5 cm dichlorvos strip (AMVAC Chemical Corp., Los Angeles, CA) placed in each trap head. Malaise samples were processed in the same manner as pan trap samples.

Traps were deployed in each plot at a given site for one week before being moved to another site, resulting in a 5 wk trapping interval. Trapping began the first week of May both years and ended Oct 21 in 2013, and Sep 17 in 2014, for a total of five and four trapping intervals per site, respectively.

2.7. Statistical analysis

All analyses were performed using SAS v. 9.3 (SAS Institute 2011). Data were tested for normality and transformed when necessary. Ash decline, based on canopy dieback assessed in summer 2014, was compared across the four plot treatments using an analysis of variance with means separations performed using Tukey's Honest Significant Difference (TUKEY). Ash decline was further compared between 'imidacloprid levels,' where plots receiving either full or half rate imidacloprid ('treated') were compared against the bio and control plots which received no imidacloprid applications ('untreated'), using a two sample t-test.

Larval emerald ash borer density was calculated for each tree ($\text{mean density}_t = \text{larvae}_{ts} / \pi d_{ts} l_{ts}$) where "t" represents individual trees, "s" debarked (odd numbered) log sections, "d" section diameter, and "l" section length. Larval densities, along with the mean number of adult beetles captured from funnel traps, were compared across treatments using an analysis of variance. Means were also compared between imidacloprid levels and between parasitoid release (bio and dual) and non-release (chem and control) plots using *t*-tests.

As measures of establishment success of the biological control agents, the number of *T. planipennisi* and *S. agrili* recovered from destructively sampled trees, *O. agrili*-parasitized eggs,

number of parasitized emerald ash borer larvae, and percentage of parasitized emerald ash borer larvae were similarly compared using an ANOVA across treatments (chem, dual, bio, untreated), imidacloprid levels (plots that received imidacloprid applications versus those that did not), and release occurrence (plots that received parasitoids versus those that did not).

Native insect abundance measured with pan and Malaise traps was calculated and richness, diversity, and evenness were derived by plot for each treatment. Richness is the total number of taxa within each sample. We used the Shannon and Simpson indices as measures of hymenopteran diversity, which utilizes the relative abundance of each taxon and the total insect abundance within a sample (Magurran, 1988). Pielou's Index of Evenness (J), which incorporates the Shannon index and taxa richness in the sample (Magurran, 1988) was calculated (Southwood and Henderson, 2009). Population parameters (abundance, diversity indices and Pielou's evenness index) were analyzed using a repeated measure mixed linear model with sample date as the repeated measure and individual plots (traps) as subjects. Least square estimates were compared to determine differences in abundance and diversity of hymenopteran guilds among treatments and between imidacloprid levels.

3. Results

3.1. Forest composition

Ash canopy dieback was higher in trees receiving full strength imidacloprid applications than those in dual treatment plots which received a half dose of imidacloprid (Table 2.A), though trees that received chemical treatment (either full or half dose) did not differ from untreated trees (Table 2.B).

3.2. *Emerald ash borer flight intercept traps*

Funnel traps captured 453 adult emerald ash borer, including the initial detections at the Fayette and Spencer sites where six and one individual, respectively, were captured. Due to the low pressure at these two sites they were excluded from analyses of treatment effects on beetle populations. Emerald ash borer capture across treatments (Table 2.A) pooled from Anderson, Henry, and Shelby ranged from 4.8 ± 2.6 per trap in dual treatment plots to 19.0 ± 13.8 in biological control plots. There was a trend towards greater abundance in untreated plots (17.4 ± 7.4) than in chemically treated plots (7.1 ± 3.5) though this difference was not significant (Table 2.B).

Larval emerald ash borer density was lower in trees felled in 2014 than from those felled in 2015 (27 vs. 54 larvae / m²) ($t_{39} = 2.46$, $p = 0.02$); therefore, analysis of treatment and treatment level effects were carried out separately for each year. In 2014 the highest density occurred in the trees felled from biological control plots, which differed from trees treated with full strength imidacloprid (Fig. 1.A.); larval density in dual and untreated control plots was intermediate. In 2014 emerald ash borer larval density was lower in trees from chemically treated plots than those from untreated plots (Fig. 1.B). In 2015 there was no significant difference in larval densities between treatments (Fig. 2.A) or between chemically treated and untreated plots (Fig. 2.B).

3.3. *Biocontrol establishment*

In 2014 a total of 106 *T. planipennisi* were recovered; 63 larvae from two emerald ash borer galleries within debarked logs and 43 adults from two separate rearing enclosures

(representing at least two parasitized emerald ash borer larvae). Three of the parasitized emerald ash borer and 96 of the *T. planipennisi* were from trees felled from biological control plots, the other parasitized emerald ash borer larva and 10 *T. planipennisi* were from a dual treatment plot. No larval *T. planipennisi* were recovered from trees felled in plots where they were not directly released. In 2015 2,339 *T. planipennisi* were recovered from 79 parasitized emerald ash borer larvae from logs that were immediately debarked (odd section logs). Due to insufficient recovery of *T. planipennisi* in the first year of this experiment only 2015 data were used to analyze the effects of plot treatments and imidacloprid levels on parasitoid establishment success.

There was no difference between plot treatments (Table 3.A), or between chemically treated and untreated plots (Table 3.B) with regard to the number of *T. planipennisi* extracted from debarked logs, total number of emerald ash borer larvae parasitized, or percent of larvae parasitized. Trees felled from release plots (biological control and dual treatments) (Table 3.C and Fig. 3.A) yielded more *T. planipennisi* than trees from non-release plots (chemical control and untreated), and there were more emerald ash borer larvae parasitized by *T. planipennisi* per tree in release plots than non-release plots ($t_{13} = 2.36$, $p = 0.03$) (Table 3.C and Fig. 3.B).

From the 15 destructively sampled trees in 2015, 70 emerald ash borer eggs were recovered from bark shavings; 14 showed signs of parasitization from *O. agrili*. There was no difference in the number of eggs between shavings taken from logs at the two heights (0.6 m and 5.5 m); therefore they were combined for further analysis. There were also no differences in the number of total eggs across the four plot treatments, between chemically treated and untreated plots, or between biological control release versus non-release plots (Table 3.A-C). Parasitization

was only observed in shavings from release plots where 28.0% of eggs were parasitized, and did not differ between chemically treated and untreated plots ($t_{13} = 0.11$, $p = 0.92$).

No *S. agrili* were recovered at any point in our study.

3.4. Native insect communities

A total of 44,205 hymenopterans were captured in pan and Malaise traps over the two year study. Pan traps yielded 3,264 hymenopterans in 2013 and 3,248 in 2014, of which ichneumonoids (Ichneumonidae and Braconidae) comprised 26.4%, non-ichneumonoid parasitoids 53.5%, Formicidae 11.1%, Apoidea 4.3%, Symphyta 3.0%, and other hymenopterans (Pompilidae, Scoliidae, Sphecidae, Tiphidae, and Vespidae) 1.8%. There were no consistent trends in total Hymenoptera abundance or in abundance of the broad taxonomic groups of hymenopterans between plot treatments. In 2013 (data not shown) more Hymenoptera were captured in dual treatment plots than chemical control plots ($F_{3,40} = 2.91$, $p = 0.05$), although there was no difference between plots which received imidacloprid and those that did not. In 2014, the greatest abundance of Hymenoptera was captured in untreated control plots and the fewest in chemical control and biocontrol plots (Table 4.A), but there was no difference between treated and untreated plots (Table 4.B). The Ichneumonoidea were unaffected by our treatments, regardless of grouping (Table 4.A and 4.B). Similarly, the non-ichneumonoid parasitoids and the bees, or non-Sphecidae Apoidea, were unaffected (Table 4.A and 4.B).

Malaise traps yielded 19,898 Hymenoptera in 2013 and in 17,795 in 2014.

Ichneumonoids comprised the majority of hymenopterans caught across years accounting for 69.3%, non-ichneumonoid parasitoids accounted for 15.4%, whereas bees (7.9%), Formicidae (5.0%), Symphyta (1.2%), and other Hymenoptera (1.2%) accounted for the remainder. There

was no difference in abundance of Ichneumonidea or bees between years; therefore those data were combined for further analysis. Plot treatment (chem, dual, bio, control) did not affect abundance of Ichneumonidea or bees (years combined) or non-ichneumonoid parasitoids in 2013 or 2014 (Table 5.A), nor did abundances differ between imidacloprid levels (Table 5.B). Similarly, neither Simpson's or Shannon's diversity indices, nor Pielou's measure of evenness, differed due to plot treatment (Table 6.A) or imidacloprid level in (Table 6.B) in either year.

4. Discussion

We tested a strategy for emerald ash borer management that combined imidacloprid applied at reduced rates with biological control releases, with the goal of prolonging ash decline to facilitate establishment of biological control agents. The effectiveness of this strategy at preventing ash mortality and reducing emerald ash borer populations was evaluated, along with the compatibility of imidacloprid applications with establishment of currently employed biological control agents. Non-target considerations are a critically important aspect of any sustainable management approach, so we also evaluated our strategies in the context of their effects on native parasitoid abundance, diversity and evenness. Finally, we evaluated how these emerald ash borer management tactics might specifically affect imperiled native pollinators, which provide critical ecosystem services (Blacquière et al., 2012; Pisa et al., 2015; Lawrence et al., 2016).

In forests where emerald ash borer was present at the onset of the study both full and reduced rates of imidacloprid were insufficient to prevent ash canopy decline. This may be partially attributable to emerald ash borer pressure and the level of tree decline at each site, which varied. It's likely that prior emerald ash borer damage to the vascular systems of trees at heavily infested sites compromised their ability to translocate imidacloprid to adequately prevent

further damage. Inadequate soil moisture may also have played a role, as this affects imidacloprid uptake (Smitley et al., 2015). Not surprisingly precipitation varied across study sites within and between years (Table 1), but precipitation was generally higher in March 2013, at 100% of normal, compared to March 2014, at ~70% of normal (www.ncdc.noaa.gov/cag/time-series/us), and this may be reflected in our results. Finally, a substantial portion of our trees were large, and the rates of imidacloprid we used in these large trees were not adequate for effective suppression of emerald ash borer (Smitley et al. 2015); trees with diameters greater than 38 cm (15 in.) have limitations in hydraulic structure affecting vascular translocation (Smitley et al., 2010a). There were many large, heavily infested ash at the Anderson, Henry and Shelby sites prior to installation of our plot treatments. However, in 2014 there were lower densities of larval emerald ash borer in trees that received imidacloprid applications, regardless of rate, compared to untreated trees and there was no difference in larval densities between dosages. This suggests that a half dose rate is a promising component of an emerald ash borer management program developed to facilitate establishment of biological control agents.

Recovery of *T. planipennisi* from trees felled in winter 2014 suggests that despite relatively low release numbers and likely suboptimal timing, parasitoids released in 2013 successfully located emerald ash borer host larvae and reproduced, though recovery was too low to evaluate the effects of our plot treatments on these early stages of parasitoid establishment. Vastly increased *T. planipennisi* recovery in 2015 was likely due to the much greater release effort during the summer of 2014 in combination with establishing populations of parasitoids deployed in 2013.

Recovery of *T. planipennisi* and *O. agrili* from trees felled in winter 2015 was higher in release plots (dual treatment and biological control) than non-release plots (chemical control and

untreated), and recovery of both species, along with the number and percent of emerald ash borer larvae parasitized by *T. planipennisi*, were similar between trees from chemically treated and untreated plots. These results demonstrate that imidacloprid applied as a soil drench can suppress larval emerald ash borer populations, yet sufficient emerald ash borer eggs and larvae persist that establishment of *T. planipennisi* and *O. agrili* are not negatively impacted, further suggesting compatibility of chemical control for emerald ash borer with these classical biological control agents.

The lack of *S. agrili* recovery in our study was not unexpected; this may reflect the relatively low release numbers (Table 1) and is also consistent with results from other areas demonstrating the difficulties in getting this species established as a biocontrol agent for emerald ash borer (Gould et al., 2015). Fortunately, a closely related congeneric, *S. galinae*, may be more amenable to establishment for emerald ash borer population regulation in the U.S., and has recently been approved for release (<https://entomologytoday.org/2016/05/26/usda-to-release-russian-wasps-against-the-emerald-ash-borer/>).

Ours was a short term, small scale study utilizing 0.04 ha plots to evaluate the compatibility of these management approaches. Unfortunately, we had no quantitative data on canopy conditions prior to implementation of our plot treatments, and because of this, attributing differences in canopy condition solely to our plot treatments, without considering site effects, is impossible. Further, emerald ash borer pressure in the surrounding forest was intense and we saw extensive canopy decline regardless of treatment, corroborating Smitley et al., 2015, who found that protecting all ash within a forest stand is much more effective than treating a smaller proportion of susceptible trees. If most or all of the trees within our larger forested tracts were

381 treated, the reduced imidacloprid rate may have been adequate to provide the same or greater
382 level of protection than did the full strength rate.

383 As the emerald ash borer invasion has progressed in the eastern U.S. several native
384 parasitoid species have been discovered utilizing *A. planipennis* (Bauer et al., 2004; Duan et al.,
385 2009; Duan et al., 2012a; Davidson and Rieske, 2015), presumably recruited from a rich
386 reservoir of North American Buprestidae (Evans, 2014; Paiero et al., 2012; Taylor et al., 2012).
387 In Kentucky where our study was conducted and where the emerald ash borer infestation is
388 spreading rapidly, there is a diverse array of native parasitoids which are being recruited from
389 their historic hosts to this novel and plentiful host (Davidson and Rieske 2015). Although the
390 ensuing ecological interactions are likely still developing and not fully understood, observed
391 levels of parasitization from these natives is low. Nevertheless, any newly implemented
392 sustainable management protocols should take these potential population regulators into
393 consideration. Therefore, we evaluated the effects of imidacloprid applications and biocontrol
394 releases on populations of these native hymenopterans. Although slight differences in
395 hymenopteran guild abundances among our plot treatments was evident, there were no consistent
396 differences across sampling approaches in abundances in total Hymenoptera, ichneumonoidea,
397 and non-ichneumonoid parasites between plots receiving imidacloprid treatment compared to
398 untreated control plots. Further, there were no differences in diversity or evenness of total
399 hymenoptera or the hymenopteran guilds investigated between plot treatments and between
400 chemically treated and untreated control plots, indicating that there was little impact of
401 imidacloprid applied as a soil drench on native hymenopteran populations. Finally and
402 importantly, we found no effects of imidacloprid applied as a soil drench for emerald ash borer
403 management on native pollinator populations. Our data suggest that judicious use of insecticides

404 provides a promising approach to facilitate establishment of biological control against invading
405 emerald ash borer populations, with little effect on non-target hymenopterans.

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Table 1. Plot treatments across five study sites in north-central Kentucky evaluating emerald ash borer management and describing i) imidacloprid applications and ii) parasitoid deployment for 2013 and 2014.

	Anderson	Fayette ²	Henry	Shelby	Spencer ³	
A. Imidacloprid applications						
2013						
Application date	15 Mar	12 Mar	21 Mar	27 Mar	14 Mar	- - -
Precipitation ¹ (cm) (deviation)	11.20 (+0.91)	13.36 (+2.97)	9.25 (-1.57)	6.27 (-4.39)	10.39 (+0.05)	- - -
2014						
Application date	21 Mar	17 Mar	20 Mar	19 Mar	14 Mar	- - -
Precipitation ¹ (cm) (deviation)	6.73 (-3.38)	7.49 (-2.74)	12.83 (+1.98)	12.98 (+2.11)	8.51 (-1.83)	- - -
ii. Parasitoid releases						
2013						Total
<i>Tetrastichus planipennisi</i>	6,296	0	5,646	6,756	0	18,698
<i>Spathius agrili</i>	0	0	0	0	0	0
<i>Oobius agrili</i>	0	0	0	0	0	0
2014						
<i>Tetrastichus planipennisi</i>	21,259	10,366	21,736	21,563	0	74,924
<i>Spathius agrili</i>	2,920	244	3,658	3,705	0	10,527

<i>Oobius agrili</i>	5,700	3,200	5,300	5,450	0	19,650
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¹Includes precipitation and deviation from normal for period one week prior and three weeks following application date, measured at nearest available weather station (KY Mesonet www.agwx.ca.uky.edu/)

²Not eligible for parasitoid releases until Jul. 15 2014

³Not eligible for parasitoid releases for the duration of the study

Table 2. Characteristics of forested plots in north-central Kentucky used to evaluate suppression approaches and incidence of emerald ash borer.

A.Treatment ^a	No. <i>Fraxinus</i> stems ¹	% <i>Fraxinus</i> stems ¹	% canopy dieback	Mean EAB
			(S.E.) in 2014	abundance (S.E.) ²
Chemical	117	22.0	44.6 (4.7) a	9.3 (6.5)
Dual	82	13.7	30.3 (3.5) b	4.8 (2.6)
Biological control	65	12.7	39.9 (3.9) ab	19.0 (13.8)
Untreated	90	17.9	34.9 (4.0) ab	16.4 (6.5)
			$F_{3,350} = 2.61; p = 0.05$	$F_{3,32} = 0.60; p = 0.62$
B. Chemically treated ^b				
Treated	199	17.6	38.7 (2.8)	7.1 (3.5)
Untreated	155	15.3	37.0 (2.9)	17.7 (7.4)

$$t_{352} = 0.43; p = 0.67 \quad t_{34}; p = 0.20$$

^aTreatments include chemical, dual, biological control and untreated. See text for full explanation

^bChemically treated refers to either Treated = full or reduced rate imidacloprid applications, or Untreated = biological control releases and untreated controls

¹*Fraxinus* stems > 2.5 cm at 1.5 m above ground level (DBH)

²Activity based on 2014 adult beetle catch in green multi-funnel traps baited with Manuka oil and hexanol lures

Table 3. Recovery in 2015 of *T. planipennisi* and *O. agrili* (mean $\pm$ s.e.) released over two years (2013 and 2014) from five sites in north-central Kentucky.

	<i>Tetrastichus planipennisi</i>			<i>Oobius agrili</i>		
	parasitoids	parasitized	% parasitized	Total	parasitized	% parasitized
A. Treatment ^a	recovered	EAB larvae	EAB larvae	EAB eggs	EAB eggs	EAB eggs
Chemical	25.5 (25.5)	3.3 (3.3)	1.5 (1.5)	1.5 (0.5)	0 (0.0)	0 (0.0)
Dual	340.3 (155.5)	29.5 (20.1)	12.0 (3.2)	5.3 (2.2)	1.7 (0.7)	32.1 (25.7)
Biocontrol	206.2 (79.3)	13.7 (5.9)	9.6 (4.2)	5.7 (1.5)	1.5 (0.9)	26.3 (9.1)
Untreated	47.2 (25.1)	10.5 (6.6)	4.4 (1.0)	4.3 (2.7)	0 (0.0)	0 (0.0)
$F_{3, 11}; p$	2.68; 0.10	0.88; 0.48	1.81; 0.20	0.57; 0.64	1.14; 0.38	3.17; 0.06
B. Chemically Treated ^b						
Treated	214.4 (115.2)	19.0 (13.2)	7.8 (3.2)	3.8 (1.5)	1.0 (0.5)	26.3 (18.9)
Untreated	126.7 (47.3)	12.1 (4.2)	6.8 (2.2)	5.1 (1.3)	0.9 (0.6)	17.6 (5.4)
$t_{13}; p$	0.84; 0.41	0.64; 0.53	0.26; 0.80	1.10; 0.29	0.11; 0.92	1.00; 0.33
C. Release Occurrence ^c						
Parasitoid release	256.5 (73.7)	19.6 (8.2)	10.5 (2.7)	5.6 (1.2)	1.6 (0.6)	28.6 (10.5)

Non-release	41.0 (17.4)	8.5 (4.8)	3.3 (0.9)	3.3 (1.8)	0 (0.0)	0 (0.0)
$t_{13}; p$	2.66; 0.02	1.13; 0.28	2.36; 0.03	0.60; 0.56	2.00; 0.07	2.61; 0.02

^aTreatments include chemical, dual, biological control and untreated. See text for full explanation

^bChemically treated refers to either Treated = full or reduced rate imidacloprid applications, or Untreated = biological control releases and untreated controls

^cRelease occurrence refers to Parasitoid release = biological and dual treatments, or Non-release = full strength imidacloprid and untreated

Table 4. Least square estimates (mean $\pm$ s.e.) of native insects captured using pan traps in 2014. Differing letters following values indicate differences significant at $\alpha = 0.05$.

A. Treatments ^a	Non-Ichneumonoid ²			
	Hymenoptera (S.E.)	Ichneumonoidea ¹ (S.E.)	parasitoids (S.E.)	Apoidea ³ (S.E.)
Chemical	9.4 (1.5) b	3.3 (0.6)	5.4 (1.3)	0.7 (0.2)
Dual	12.7 (1.1) ab	3.9 (0.6)	8.4 (1.4)	0.4 (0.2)
Biocontrol	11.6 (1.1) b	4.0 (0.6)	7.2 (1.4)	0.4 (0.2)
Untreated	16.5 (1.1) a	4.5 (0.6)	11.0 (1.4)	0.7 (0.2)
$F_{3, 56}; p$	3.55; 0.02	0.85; 0.47	1.57; 0.12	1.12; 0.35
B. Chemically treated ^b				
Treated	11.0 (1.2)	3.6 (0.4)	6.9 (1.0) b	0.6 (0.2)
Untreated	14.0 (1.2)	4.2 (0.4)	9.1 (1.0) a	0.7 (0.2)
$t_{58}; p$	1.82; 0.07	1.16; 0.25	3.02; 0.04	0.59; 0.55

^aTreatments include chemical, dual, biological control and untreated. See text for full explanation

^bChemically treated refers to either Treated = full or reduced rate imidacloprid applications, or Untreated = biological control releases and untreated controls

¹Ichneumonoidea includes Braconidae and Ichneumonidae

²Non-ichneumonoid parasitoids include superfamilies: Ceraphronoidea, Chalcidoidea, Chrysidoidea, Cynipoidea, Diaprioidea, and Platygastroidea

³Apoidea sans Sphecidae

Table 5. Least square estimates (mean $\pm$ s.e.) of hymenopteran guilds captured in Malaise traps in 2013 and 2014.

Treatment ^a	Ichneumonoidea ¹	Non ichneumonoid ²		Apoidea ³
		parasitoids (2013)	parasitoids (2014)	
Chemical	50.1 (3.2)	9.0 (1.2)	11.8 (2.3)	5.0 (1.5)
Dual	53.2 (3.3)	10.1 (1.2)	12.9 (2.4)	4.5 (1.5)
Biocontrol	50.0 (3.2)	10.7 (1.2)	12.9 (2.3)	7.0 (1.5)
Untreated	53.7 (3.2)	11.5 (1.2)	12.6 (2.4)	7.2 (1.5)
$F_{3, 56}; p$	0.39; 0.76	0.77; 0.52	0.23; 0.87	0.88; 0.46
Chemically treated ^b				
Treated	52.6 (2.5)	9.5 (0.8)	11.8 (2.3)	4.7 (1.0)
Untreated	54.1 (2.5)	11.1 (0.8)	11.8 (2.3)	7.1 (1.0)
$t_{58}; p$	0.43; 0.67	1.31; 0.20	0.60; 0.55	1.63; 0.11

^aTreatments include chemical, dual, biological control and untreated. See text for full explanation

^bChemically treated refers to either Treated = full or reduced rate imidacloprid applications, or Untreated = biological control releases and untreated controls

¹Ichneumonoidea includes Braconidae and Ichneumonidae

²Non-ichneumonoid parasitoids include superfamilies: Ceraphronoidea, Chalcidoidea, Chrysidoidea, Cynipoidea, Diaprioidea, and Platygastroidea

³Apoidea sans Sphecidae

Table 6. Indices of richness and diversity (mean $\pm$ s.e.) for hymenopterans captured in Malaise traps in 2013 and 2014

	Simpson's	Simpson's	Shannon's	Shannon's	Pielou's J	Pielou's J
	(2013)	(2014)	(2013)	(2014)	(2013)	(2014)
A. Treatment ^a						
Chemical	0.43 (0.02)	0.49 (0.02)	0.75 (0.03)	0.85 (0.04)	0.60 (0.02)	0.63 (0.02)
Dual	0.43 (0.02)	0.46 (0.02)	0.79 (0.03)	0.80 (0.04)	0.58 (0.02)	0.60 (0.02)
Biocontrol	0.45 (0.02)	0.52 (0.02)	0.81 (0.03)	0.89 (0.04)	0.57 (0.02)	0.64 (0.02)
Untreated	0.43 (0.02)	0.50 (0.02)	0.77 (0.03)	0.85 (0.04)	0.57 (0.02)	0.66 (0.02)
$F_{3, 56}; p$	0.15; 0.93	1.20; 0.30	0.78; 0.51	1.10; 0.36	0.25; 0.86	0.92; 0.44
B. Chemically Treated ^b						
Treated	0.43 (0.01)	0.47 (0.02)	0.77 (0.03)	0.82 (0.02)	0.59 (0.02)	0.62 (0.02)
Untreated	0.44 (0.01)	0.51 (0.02)	0.79 (0.02)	0.87 (0.02)	0.57 (0.02)	0.66 (0.02)
$t_{58}; p$	0.59; 0.56	1.58; 0.12	0.60; 0.55	1.45; 0.15	0.56; 0.58	1.48; 0.14

^aTreatments include chemical, dual, biological control and untreated. See text for full explanation

^bChemically treated refers to either Treated = full or reduced rate imidacloprid applications, or Untreated = biological control releases and untreated controls

1 **Figure captions**

2 **Fig. 1.** Larval emerald ash borer density (mean $\pm$ s.e.) within ash felled in 2014 between
3 A. plot treatments ('Chem' – imidacloprid only, 'Dual' – imidacloprid + parasitoid
4 releases, 'Bio' – parasitoid releases only, and 'Control' – no treatment), and between B.
5 treatment levels ('Treated' – plots treated with imidacloprid, including 'Chem' + 'Dual',
6 and 'Untreated' – plots with no chemical treatment, including 'Bio' and 'Control'), and
7 within ash felled in 2015 between C. plot treatments and between D. treatment levels.

8 **Fig. 2.** The number of A. larval *Tetrastichus planipennisi* recovered and B. emerald ash
9 borer larvae parasitized within ash felled in winter 2015 from parasitoid release
10 (biological control and dual treatment) plots and non-release (chemical and untreated)
11 plots.

Fig. 1.

14 **Fig. 2**

Davidson & Rieske

15