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Monitoring field establishment of the emerald ash borer biocontrol agent *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae): Sampling methods, sample size, and phenology

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HIGHLIGHTS

- Yellow pan traps and bark-sifting had higher probabilities of recovering *O. agrili*.
- Sampling trees with fresh woodpecker feeding holes improved *O. agrili* recovery.
- Sampling 10 trees per site maximized benefit:cost ratios for sampling precision.
- Most *O. agrili* adults were active between 433 and 1068 GDD (base 10 °C).

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ABSTRACT

Monitoring *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), an egg parasitoid being released for biological control of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), is challenging due to its small size and the cryptic placement of host eggs. We compared four *O. agrili* recovery methods: 1) rearing adults from bark (bark rearing); 2) sifting parasitized eggs from bark (bark sifting); 3) placing sentinel EAB eggs in screened envelopes on ash trees (sentinel eggs); and, 4) placing yellow pan traps on ash trees to capture adult parasitoids. In 2016, we sampled 40 trees within 0.25-ha-plots at each of 4 sites in Michigan with each recovery method. In 2017 and 2018, methods were applied to 10 trees within 0.25-ha-plots at each of 3 sites. Sentinel eggs were not included in 2018. Yellow pan traps and bark sifting recovered *O. agrili* in all sites and years, had higher percentages of *O. agrili*-positive trees, and required fewer trees sampled for > 95% probability of *O. agrili* recovery compared to bark rearing and sentinel eggs. When sampling only trees with fresh woodpecker-feeding holes, a sign of recent EAB attack, the probability of *O. agrili* recovery increased substantially for bark sifting and bark rearing, increased slightly for yellow pan traps, but decreased for sentinel eggs compared to sampling all trees. Peak recovery using yellow pan traps and sentinel eggs occurred between 400 and 1200 growing degree days (base 10 °C, January 1 start date), revealing when most *O. agrili* adults were active. The type of information each of these parasitoid-recovery methods provides and their relative efficiencies are discussed.

1. Introduction

Oobius agrili Zhang and Huang (Hymenoptera: Encyrtidae) is a classical biological control agent of emerald ash borer (EAB), *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), first released into the U.S. in 2007 (Bauer et al., 2015; Duan et al., 2018). In its native range in northeastern China, *O. agrili* is an important natural enemy of EAB, with mean parasitism rates up to 32–44% in some areas (Liu et al., 2007; Wang et al., 2016). A solitary, multivoltine egg parasitoid of EAB, *O. agrili* overwinters as diapausing larvae inside host eggs. In North America, *O. agrili* reproduces by thelytokous parthenogenesis and males are absent, although males were reported from China (Zhang et al.,

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2005).

As of 2020, almost 2 million *O. agrili* have been released in 27 states and the District of Columbia in the U.S., and 3 Canadian provinces, and its establishment has been confirmed in 14 states and 2 Canadian provinces (MapBioControl, 2020). EAB egg parasitism rates as high as 40% have been reported from some of the earliest release sites in Michigan (Abell et al., 2014). As the releases of *O. agrili* expand in North America, reliable methods for monitoring establishment and abundance are essential for evaluating its efficacy as an EAB biocontrol agent. However, *O. agrili*'s small size (ca. 1-mm long) makes adults particularly challenging to recover in the field. Immature *O. agrili* inside EAB eggs are also difficult to recover because EAB females lay their eggs in crevices or between layers of bark (Abell et al., 2014; Liu et al., 2007; Wang et al., 2010).

The Emerald Ash Borer Biological Control Release and Recovery Guidelines (referred to hereinafter as *EAB Biocontrol Guidelines*) (USDA–APHIS/ARS/FS, 2019) were developed to provide researchers and managers with methods for releasing and recovering EAB biological control agents, including *O. agrili*. The EAB Biocontrol Guidelines document is available on-line and updated periodically as new research findings come to fruition, incorporating the most current information available to maximize release and recovery success. The three sampling methods recommended in the EAB Biocontrol Guidelines for *O. agrili* recovery include a minimum of: 1) 15 yellow pan traps for capturing adults collected monthly during spring–fall; 2) 10 bark samples sheered from the outer bark of ash trees and sifted for parasitized eggs (bark sifting), or 3) 30 bark samples sheered from the outer bark of ash trees for rearing adults (bark rearing). Although these methods are known to recover *O. agrili* in the field, their efficiencies have not been directly compared.

Yellow pan traps are frequently used to collect hymenopteran parasitoids and Noyes (1990) found them to be more effective for sampling adult Encyrtidae and other Chalcidoidea, compared to other methods such as canopy fogging, malaise trapping, window pane trapping, and sweep netting. The yellow pan traps used to capture *O. agrili* adults consist of yellow plastic bowls, filled with an insect-trapping solution, which are attached to lower trunks of ash trees (USDA–APHIS/ARS/FS, 2019). Trap samples are sorted under a dissecting microscope, and suspect individuals are collected and confirmed using morphological characters. Parisio et al. (2017) recovered *O. agrili* with yellow pan traps at release sites in New York, and recoveries of *O. agrili* at several additional locations in the U.S. are reported in MapBioControl (2019). However, Jones et al. (2019) recovered no *O. agrili* with yellow pan traps the same year *O. agrili* was released at their study sites. Limited *O. agrili* dispersal ability, coupled with lack of olfactory attractants, likely minimizes the effective trapping range of yellow pan traps.

Field collection of host eggs is frequently used for surveying egg parasitoids that attack exposed eggs that are relatively easy to locate such as *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae) (Jones et al., 2019) and *Anas tristis* (DeGreer) (Hemiptera: Coreidae) (Cornelius et al., 2016). EAB eggs parasitized by *O. agrili* have been recovered by visual inspection of host-tree bark in the field (Abell et al., 2014; Duan et al., 2012; Jennings et al., 2018; Liu et al., 2007; Wang et al., 2016). However, this method is challenging because EAB eggs are usually hidden in crevices or between layers of bark, requiring careful removal of the outer bark scales without accidentally dislodging EAB eggs. After bark scales are removed, detection of EAB eggs are subject to the inspectors' visual acuity and natural lighting and weather conditions in the field (Abell et al., 2014).

The bark-sifting method is a modified visual-inspection method that entails sheering off the outer layer of bark from EAB-infested ash trees with a draw knife, and collecting the bark with a plastic drop cloth or container (Abell et al., 2014). In the laboratory, bark-sifting samples are stored and dried in paper bags, then shaken and sifted with a soil sieve to dislodge and separate smaller debris, including EAB eggs, from larger bark pieces. Using a dissecting microscope with bright lighting, the EAB eggs are then sorted and collected from the sifted bark debris, and their status determined (i.e., hatched or parasitized) (Minnesota Department of Agriculture, 2019; USDA–APHIS/ARS/FS, 2019). Abell et al. (2014) found the bark-sifting method more effective at recovering EAB eggs compared to visually inspecting for EAB eggs in the field. By contrast, Jennings et al. (2018) found visual inspection in the field more effective compared to bark sifting; however, they sampled one-fifth of the surface area recommended by the EAB Biocontrol Guidelines for the bark-sifting method.

Oobius agrili can also be recovered by rearing adults from logs or bark collected from EAB-infested trees. Ash bark or logs must be sampled during winter or early spring after diapausing *O. agrili* larvae are exposed to sufficient cold to terminate diapause (Duan and Larson, 2019; Petrice et al., 2019). The ash materials are then reared indoors in containers equipped with emergence cups for collecting emerging adult parasitoids. Parisio et al. (2017) recovered more *O. agrili* by rearing adults from logs cut from EAB-infested trees compared to captures of adults in yellow pan traps at the same release sites. Also, Abell et al. (2014) used the bark-rearing method to rear *O. agrili* adults from bark samples prior to sifting, sorting, and collecting EAB eggs using the bark-sifting method.

The use of sentinel host eggs is another effective egg parasitoid sampling method (Cornelius et al., 2016; Herlihy et al., 2016; Herz et al., 2007; Moya-Raygoza et al., 2012). Volatiles from host eggs can attract some egg parasitoid species (Michereff et al., 2016; Vinson, 1998); potentially making sentinel eggs more effective than other sampling methods when host densities are low. Although the EAB Biocontrol Guidelines do not recommend sentinel eggs for sampling *O. agrili*, they have been used to successfully detect *O. agrili* in the field. For example, Duan et al. (2011) placed sentinel EAB eggs under bark flaps and recovered *O. agrili* at some study sites. Small ash bolts on which EAB females had oviposited eggs in the laboratory (i.e., sentinel egg bolts) were also used successfully to detect *O. agrili* in the field (Duan et al., 2012, Abell et al., 2016). However, Parisio et al. (2017) recovered no *O. agrili* using sentinel egg bolts or sentinel eggs in plastic cups, even though *O. agrili* was reared from ash logs and collected in yellow pan traps at the same study sites. In addition to rearing and maintaining EAB adults for oviposition, preparing sentinel egg bolts is time-consuming and EAB oviposition on sentinel egg bolts is highly variable (Abell et al., 2016; Duan et al., 2012; Parisio et al., 2017). Also, exposed EAB sentinel eggs often suffer high predation rates, possibly by ants, while in the field (Duan et al., 2012). To reduce this problem, Jennings et al. (2014a,b) developed a simple solution by cutting the desired number of EAB eggs from coffee-filter papers on which EAB had oviposited and placing them in screened envelopes with openings that allowed *O. agrili* to enter but deterred predators (i.e., sentinel EAB eggs in screened envelopes or "sentinel eggs"). When sentinel eggs were placed on trees where *O. agrili* were released, EAB eggs within the envelopes were parasitized by *O. agrili* (Jennings et al., 2014a).

Although each of the four sampling methods described above has successfully recovered *O. agrili* in the field, it is unclear which method is optimal when considering the type of data provided by each method, as well as their effectiveness and logistical requirements. The number of samples needed is also unknown for each method to confidently determine presence and abundance of *O. agrili*, as well as optimal deployment time for methods that target *O. agrili* adults. To address these questions, we conducted studies in 2016–2018 with the following objectives: 1) compare bark rearing, bark sifting, sentinel eggs, and yellow pan trap methods for recovering *O. agrili* in the field; 2) estimate the number of samples required for each method to confidently survey for *O. agrili*; and 3) determine the optimal seasonal deployment of sample methods that target recovery of adult *O. agrili* (i.e., yellow pan traps and sentinel eggs). Results from this study will allow managers and researchers to select the most appropriate sampling method, sample size, and sampling period for recovering *O. agrili* in the field.

2. Materials and methods

2.1. Sampling methods

Each yellow pan trap consisted of a 355 mL (18-cm dia) yellow plastic bowl (NationWideParty.com) attached to a metal shelf bracket (15-cm vertical length × 20-cm horizontal length), which was then secured with wood screws to an ash tree approximately 1.75 m above the ground (Fig. 1A). Yellow pan traps were placed on the south side of ash trees with the assumption that direct sunlight would increase their visual attraction to parasitoids. We nested a second yellow bowl inside the first bowl to capture insects and allow easy removal of samples. A solution of 50% food-grade propylene glycol (ChemWorld.com, Taylor, MI) and 50% water was added to the second bowl to kill and preserve insects entrapped in the bowl. We added a small amount (ca. 10 mL per L) of unscented dish soap to the solution to reduce surface tension. Detailed instructions on yellow pan trap construction and sampling are given in the EAB Biocontrol Guidelines (USDA–APHIS/ARS/FS, 2019). When collecting from traps, we poured trap samples through paint strainers to separate insects from the trapping solution, and then froze insects for later processing. We inspected the trap contents in the laboratory under a dissecting microscope for suspect *Oobius* adults, and confirmed identifications of *O. agrili* using the key and descriptions in Triapitsyn et al. (2015).

Sentinel eggs were produced by rearing EAB adults at 25 °C and allowing them to oviposit on filter papers as described by Rutledge and Keena (2012). We removed filter papers with EAB eggs from rearing cups every 2–3 d and placed them in 10 °C within 24 hr to retard egg development for a maximum of 3 d until deployment. This ensured that all EAB eggs had developed for 1–4 d before deployment and would be suitable for *O. agrili* attack. EAB eggs older than 8 d held at 25 °C are rarely attacked by *O. agrili* (Duan et al., 2014). We cut and placed sections of filter papers that contained a total of 10 EAB eggs in 4 × 5 cm envelopes constructed from nylon screening (Component Supply, Part no. U-CMN-790, www.componentsupplycompany.com) with 0.79-mm openings. Edges of envelopes were stapled closed. We stapled screen envelopes with sentinel eggs approximately 1.75 m above the ground on the north side of the same trees that received yellow pan traps (Fig. 1B).

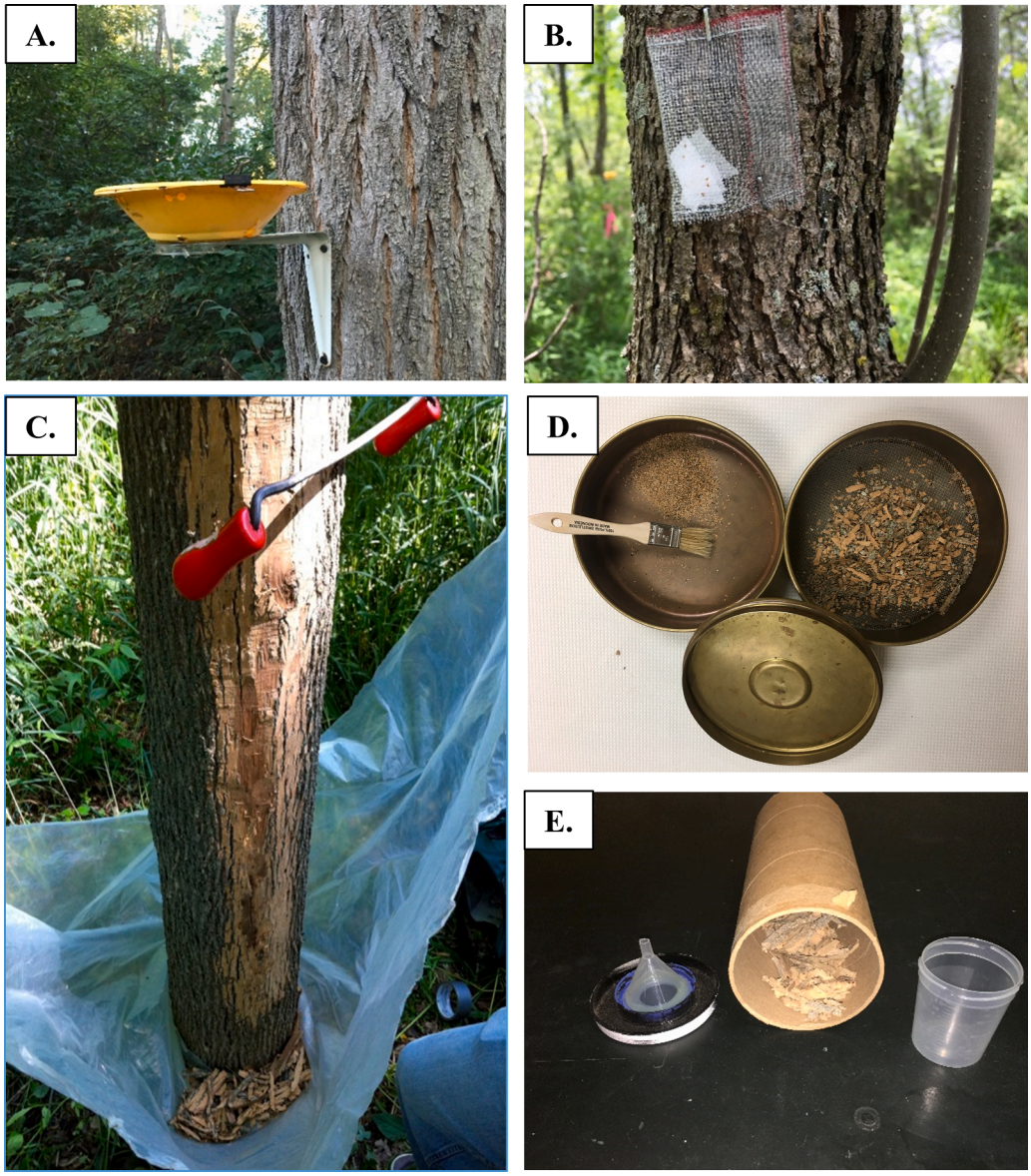

Fig. 1. Photos of different methods for sampling *O. agrili*: A) yellow pan trap; B) EAB eggs in screened envelopes (sentinel eggs); C) bark sheering; D.) sifting sheered bark for EAB eggs (bark sifting); sheered bark in rearing container (bark rearing). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Sentinel eggs were placed on the north side of trees to minimize potential temperature and desiccation effects of direct sunlight on EAB eggs (Parisio et al., 2017). After collection, we stored sentinel eggs at 25 °C for a maximum of 7 d until we inspected them for *O. agrili* parasitism. Signs and symptoms of parasitism included: chorion darkened, parasitoid egg stalk present, distorted aeropyles, and developing parasitoid visible through chorion (Minnesota Department of Agriculture, 2019; USDA–APHIS/ARS/FS, 2019). We placed parasitized eggs in 50 mm × 9 mm Petri dishes with friction-fitted lids (Fisher Scientific, catalog number 08–757- 105) to allow *O. agrili* adults to eclose.

We collected bark samples for rearing and sifting by sheering off the outer corky bark-layer with a draw knife from a 1000 cm^2 area on the lower trunk of ash trees (Fig. 1C). When possible, bark samples were taken 1.25–2.0-m above the ground from the south or west side of ash trees. However, for some situations we shifted the aspect and height if bark in the preferred position was not suitable for sampling (i.e., portion of the tree was dead, had heavy scarring, or corky bark was removed during previous sampling). We tightly wrapped a clear plastic tarp around the tree trunk with the unattached edges raised to capture the sheered bark. Bark samples were transferred from the plastic tarp to paper bags, and the bag top was folded over and closed with binder clips. Detailed methods for bark sampling are given in the EAB Biocontrol Guidelines.

Within three days of collection, we placed the bark samples into rearing containers to allow adult *O. agrili* to develop and emerge. The rearing containers consisted of 10-cm-dia cardboard tubes that were 25-cm-long (Fig. 4E). We placed plastic plugs, painted black (Rust-Oleum® Paint for Plastic, Rust-Oleum® Corporation, Vemon Hills, IL) to reduce light transmission, in the ends of tubes. We drilled a 3.8-cm-hole in the center of one of the plastic plugs for each container. The same size hole was drilled in a 133-mL plastic specimen cup lid. The top surface of the specimen cup lid was attached to the plastic plug using a hot glue gun after both holes were aligned. Then we glued the large end of a clear plastic funnel (Maryland Plastics, Federalsburg, MD, Part # L-1000PP) that was slightly smaller than the diameter of the specimen-cup lid to the inside of the specimen cup lid. The inside diameter of the funnel stem was 7 mm. We placed the specimen cup bottom over the funnel and screwed it into the specimen-cup lid. A supply list and instructions for constructing rearing containers are given in the EAB Biocontrol Guidelines. A small amount of honey was streaked onto the inside of the cup as food for emerging parasitoids. We reared bark samples for a minimum of eight weeks to allow diapausing *O. agrili* larvae to develop to adults and emerge. Adult parasitoids were collected every other day from the emergence cups and placed in 70% ethanol for identification at a later date.

After 60 d in rearing tubes, we placed each bark-sifting sample in a No. 14 soil sieve with a tight-fitting lid (Fig. 1C), and shook it aggressively for a 2-min-period, alternating from side-to-side- to up-and-down-motions every 15 s. We filled the soil sieve no more than half full for each two-minute shake period to increase the likelihood of dislodging eggs during the shaking process. The shaking process was repeated until the entire sample had been shaken and sifted. We removed the bark debris captured in the bottom of the sieve and placed it in a small plastic container with a screw top. Static reducing spray (StaticGUARD®, B&G Foods, Parsippany, NJ) was applied as needed on the sieve and plastic containers to reduce static electricity. We sorted the bark debris in a porcelain tray (to reduce static) under a dissecting microscope to identify and remove EAB eggs (Fig. 4D). Then we determined the fate of each EAB egg by carefully examining for signs and symptoms of parasitism. EAB eggs that hatched usually contained light-colored frass and a jagged, elongate exit hole on their bottom surface where the EAB larva had exited the egg and entered the tree. Eggs parasitized by *O. agrili* were usually darker, contained dark clumps of parasitoid meconium, and a round exit hole on the side or top (Minnesota Department of Agriculture, 2019; USDA–APHIS/ARS/FS, 2019). Other signs and symptoms of *O. agrili* attack included a parasitoid egg stalk and distorted aeropyles on the EAB egg surface from the developing parasitoid enlarging the egg during development. Occasionally, eggs contained dead EAB larvae or *O. agrili* adults, pupae, or larvae.

2.2. Study sites

We conducted studies in 2016–2018 at four sites in Michigan where *O. agrili* is established. Three of these sites were in south central Lower Michigan [Gratiot-Saginaw (near Ithaca; lat. 43.2337, long. − 84.4477); Legg Park (near Okemos; lat. 42.69403, long. − 84.3822), and Harris Nature Center (near Okemos; lat. 42.6965, long. − 84.3752)] and one site in northwest Lower Michigan [Eastport (near Eastport; lat. 45.1139, long. − 85.3324)] (Supplemental Table A1). All sites were dominated by ash trees and current EAB populations were moderate to high. *Oobius agrili* releases began in 2008 at Harris Nature Center, and establishment was confirmed in 2012. No releases were conducted at Legg Park but establishment was confirmed in 2013, presumably spreading from Harris Nature Center (located 1 km away). *Oobius agrili* releases at Gratiot-Saginaw began in 2009, and establishment was confirmed in 2010. Releases at Eastport were conducted in 2014, and establishment was confirmed in 2015.

2.3. 2016 Sampling

In 2016, all four sampling methods were compared at Eastport, Gratiot-Saginaw, Harris Nature Center, and Legg Park. At each site, 40 trees that were ≥ 10-cm-DBH (diameter at breast height) were sampled within a ca. 0.25 ha area. Trees < 10-cm-DBH were not sampled because: a) EAB attack densities are usually higher on larger trees; b) bark surface area for bark rearing and bark sifting sampling might be insufficient; and c) bark of larger trees has more texture, which provides more oviposition sites for EAB and is more conducive to sheering bark for bark rearing and bark sifting sampling. For each tree, we recorded the following signs and symptoms of EAB attack: a) number of fresh woodpecker-feeding holes on the lower 4 m of the tree bole, b) number of epicormic shoots on the lower 4 m of the tree bole, c) number of EAB-adult exit holes on the lower 2 m of the tree bole, and d) canopy dieback estimated to the nearest 10%. These data were recorded pre-season (i.e., in late spring 2016 when yellow pan traps and sentinel eggs were deployed), and post-season (i.e., winter or early spring 2017 when bark-sifting and bark-rearing samples were collected), with the exception of canopy dieback that could not be recorded in winter or early spring because leaves were not present. Tree condition ranged from severe dieback with multiple fresh woodpecker-feeding holes and epicormic shoots, to apparently healthy with no evidence of EAB attack (Supplemental Table A1). All four sampling methods were applied to each tree. Yellow pan traps and sentinel eggs were deployed 24–27 May 2016 and removed 26–30 September 2016 at Gratiot-Saginaw, Harris Nature Center, and Legg Park; and deployed 10 June 2016 and removed 4 October 2016 at Eastport. Every two weeks, yellow pan trap samples were collected and bowls refilled with trapping solution, and the sentinel eggs were removed and replaced. Bark samples (used for bark rearing and bark sifting) were collected 10–13 February 2017 at Gratiot-Saginaw, Harris Nature Center, and Legg Park; and 11 May 2017 at Eastport.

2.4. 2017 and 2018 sampling

In 2017, all four sampling methods were compared at Gratiot-Saginaw, Harris Nature Center, and Legg Park. We arbitrarily selected 10 live ash trees that showed signs and/or symptoms of EAB attack within the same plots established in 2016. All four sampling methods were applied to each tree. Yellow pan traps and sentinel egg sampling were conducted 17–18 May through 28–29 September 2017. Bark samples were collected 7–9 May 2017.

In 2018, only bark sifting, bark rearing, and yellow pan trap methods

were compared at Gratiot-Saginaw, Harris Nature Center, and Legg Park. Sentinel eggs were omitted because adequate numbers of lab-reared EAB eggs were not available. Similar to 2017, 10 live trees that showed signs and/or symptoms of EAB attack were arbitrarily selected within the plots. Bark sifting, bark rearing, and yellow pan trap sampling methods were applied to each tree. Yellow pan trap sampling was conducted 17–18 May 2018 through 28 September–1 October 2018. Bark sifting and bark rearing samples were collected 15–16 May 2019.

2.5. Data analyses

2.5.1. Comparison of sample methods

Oobius agrili individuals recovered, referred to as "*O. agrili* recoveries", from each sample tree were calculated as the total annual adult yellow pan trap captures, total annual parasitized sentinel eggs, total bark reared adults, and total sifted parasitized EAB eggs. In addition, each tree was classified as *O. agrili*-positive for a particular sampling method if at least one *O. agrili* was recovered at any time throughout each sample year for each respective sampling method (i.e., yellow pan traps and bark rearing = *O. agrili* adults; bark sifting and sentinel eggs = parasitized EAB eggs). The total number of *O. agrili* recoveries and positive trees for the four sampling methods were analyzed with a generalized linear mixed model (PROC GLIMMIX) using SAS 9.4 for Windows (SAS, 2012). *Oobius agrili* recoveries were analyzed with a negative binomial distribution and a log link function because variances were considerably larger than the means (PROC UNIVARIATE), suggesting data overdispersion. Data for *O. agrili* positive trees were analyzed with a binary distribution and a logit link function. Each sample year was analyzed separately. For 2016 data, a multi-location complete block model was used for comparisons with treatment as a fixed effect and site, sample tree within site, and the interaction of site × treatment as random effects. Because fewer trees and fewer sites were sampled in 2017 and 2018, convergence issues were encountered with the nobound option in the model. Taking out this option did not allow negative intra-class correlations of some random effects yielding different denominator degrees of freedom depending on year and response variable used. Degrees of freedom were calculated using the Kenward-Rogers degrees of freedom method and rounded to the nearest whole number for ease of reporting. Residuals were evaluated for homogeneity of variance via the Levene's test and in all models and years this assumption was met. Treatment least squares means that were significantly different ($P \leq 0.05$) were separated with Tukey-Kramer means comparison procedure.

2.5.2. Estimation of sample size

We conducted bootstrap sampling using data collected in 2016, when we intensively over-sampled ash trees ≥ 10-cm-DBH within 0.25 ha plots at each site, to estimate the probability of recovering *O. agrili* and the precision of *O. agrili* recoveries as a function of number of trees sampled for each sampling method. First, bootstrap sample sizes of 2–40 trees were each iterated 2000 times for each sample method within each site using PROC SURVEYSELECT (SAS, 2012). After 2000 iterations, we noticed stability in the probability estimates and standard errors, so additional iterations were not warranted. The percentage probability of at least one tree positive for *O. agrili* was calculated for each bootstrap sample size for each sample method within each site (i.e., number of iterations that recovered *O. agrili*/total bootstrap iterations). We then fitted a four-parameter Weibull probability function to the percentage probability as a function of number of trees sampled from all four sites for each of the sampling methods using the "drc" package in R (R Core Team, 2018; Ritz et al., 2015) to estimate probability of recovery and confidence intervals as sample size increased. The coefficient of variation was calculated for the mean number of *O. agrili* recoveries from each bootstrap iteration within each bootstrap sample size from each site and sample method as an estimate of change in precision as a function of trees sampled (Stanovick et al., 2002). Next, a Pearson correlation analysis (PROC CORR; SAS, 2012) was used to determine which signs or symptoms of EAB attack recorded during pre-season (late spring 2016) and post-season (late winter/early spring 2017) were correlated with *O. agrili* recoveries for each sampling method in 2016. The bootstrapping procedure for sampling all trees as described above was repeated but included only trees with the sign or symptom of EAB attack that was most correlated with *O. agrili* recoveries. Probability curves were fitted to percent probability of recovery and coefficients of variation were calculated for number of recoveries for these bootstrap data.

2.5.3. Sampling period

We obtained growing degree days (GDD_{10}) calculated using the Baskerville-Emin method (base 10 °C; start date = January 1) from Michigan State University Enviroweather (2019) for weather stations nearest to each site for sentinel egg collection dates in 2016 and 2017, and yellow pan traps in 2016, 2017, and 2018. Weather stations were within 10 km of each study site. We calculated cumulative sentinel egg and yellow pan trap *O. agrili* recoveries as a function of GDD_{10} to determine the range of GDD_{10} when *O. agrili* adults were active. Mean GDD_{10} when: 1) *O. agrili* was first recovered, 2) maximum number (i.e., peak) was recovered, and 3) cumulative recovery reached ≥ 95%, were calculated for sentinel eggs and yellow pan traps to estimate optimal sampling time.

3. Results

3.1. Comparison of sample methods

In 2016, each of the four sampling methods recovered *O. agrili* (Table 1). Furthermore, all methods recovered *O. agrili* at each site, with the exception of bark rearing at Harris Nature Center. Parasitized eggs with exit holes were recovered at Harris Nature Center using the bark-sifting method, but no overwintering *O. agrili* were present in these eggs (Table 1). Overall, mean *O. agrili* recoveries per tree were very low and varied significantly among sampling methods ($F = 10.33$; df = 3, 10; $P = 0.0024$; Fig. 2). Mean *O. agrili* recoveries per sample tree were highest for yellow pan traps (mean ± se per tree = 0.52 ± 0.29) and bark sifting (0.32 ± 0.18), and lowest for bark rearing (0.08 ± 0.05) and sentinel eggs (0.08 ± 0.05; Fig. 2). Percentage of *O. agrili* positive trees also varied significantly among treatments in 2016 ($F = 7.95$; df = 3, 10; $P = 0.0057$, Fig. 2). Yellow pan traps had the highest percentage of *O. agrili* positive trees (33.8 ± 13.6%), and bark rearing (6.9 ± 4.1%) and sentinel eggs (4.8 ± 4.1%) had the lowest percentages (Fig. 2). Percentage of *O. agrili* positive trees were intermediate for bark sifting (23.0 ± 10.8%).

In 2017, all four sampling methods recovered *O. agrili* from the three study sites, with the exception of bark rearing at Gratiot-Saginaw (Table 1). Mean *O. agrili* recoveries per tree varied significantly among sampling methods ($F = 4.83$; df = 3, 89; $P = 0.0037$; Fig. 2) and overall recoveries were very low (Table1). Mean *O. agrili* recoveries were significantly higher for yellow pan traps (0.93 ± 0.53 per tree) compared to bark rearing (0.08 ± 0.07). Mean recoveries for sentinel eggs (0.44 ± 0.27) and bark sifting (0.33 ± 0.20) were intermediate between yellow pan traps and bark rearing. Mean percentages of *O. agrili* positive trees also varied significantly among methods ($F = 4.43$; df = 3, 104; $P = 0.0057$; Fig. 2). Yellow pan traps had a significantly higher percentage *O. agrili* positive trees (53.1 ± 18.3%) compared to sentinel eggs (14.4 ± 10.1%) and bark rearing (7.8 ± 6.5%) samples. The mean percentage of positive trees was intermediate for bark sifting (22.1 ± 13.3%).

In 2018, *O. agrili* was recovered with all three sampling methods (bark rearing, bark sifting, and yellow pan traps) at all sites, and overall *O. agrili* recoveries and positive trees were higher compared to 2016 and 2017. Mean *O. agrili* recoveries varied significantly among sampling methods ($F = 10.11$; df = 2, 83; $P = 0.0001$). *Oobius agrili* recoveries were significantly higher for yellow pan traps (2.22 ± 0.59 per tree) and

Table 1
Total number of *O. agrili* recoveries and positive trees for four sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs; yellow pan traps) at four sites (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) for three years. Eastport was only sampled in 2016. Number of trees sampled at each site was 40 in 2016 and 10 in 2017 and 2018.

Year	Site	Sampling method							
		Bark rearing adults		Bark sifting eggs		Sentinel eggs		Yellow pan traps	
		Total recovered	No. positive trees	Total recovered	No. positive trees	Total recovered	No. positive trees	Total recovered	No. positive trees
2016	Eastport	34	11	95	18	13	5	272	33
	Gratiot-Saginaw	11	3	33	14	5	3	31	8
	Harris Nature Center	0	0	8	3	5	2	10	7
	Legg Park	4	4	33	9	5	2	49	10
2017	Gratiot-Saginaw	0	0	4	2	9	2	5	3
	Harris Nature Center	1	1	4	1	1	1	25	4
	Legg Park	4	2	9	4	14	4	32	9
2018	Gratiot-Saginaw	5	3	37	9	NA	NA	19	7
	Harris Nature Center	4	1	9	4	NA	NA	27	8
	Legg Park	3	3	31	7	NA	NA	32	4

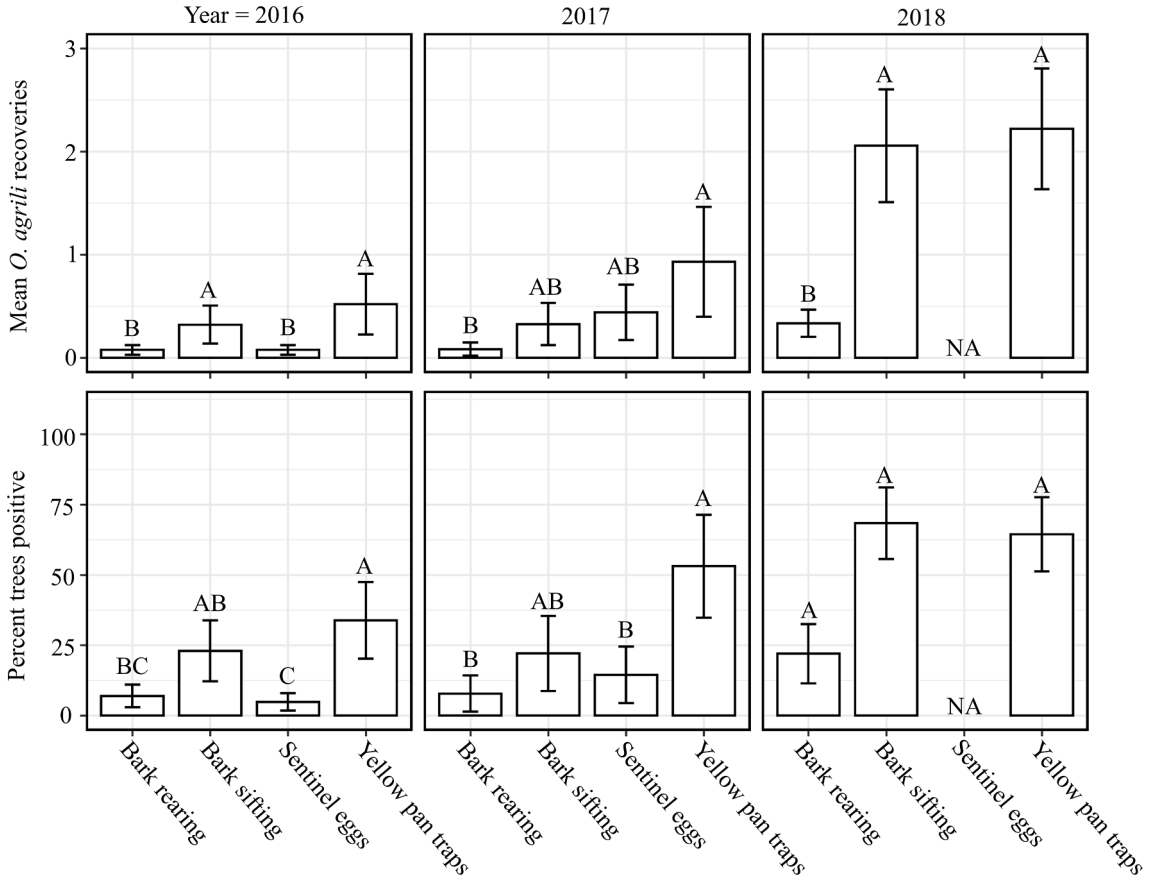

Fig. 2. Mean number of recoveries (±se) (top row) and mean percentage of positive trees (±se) (bottom row) for *O. agrili* by sampling method (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps) for three sample years. Means with different letters within each year and row are significantly different ($P \leq 0.05$; Tukey-Kramer means separation). N = 40 (2016) and N = 10 (2017 and 2018).

bark sifting (2.06 ± 0.55) methods compared to the bark rearing (0.33 ± 0.13) (Fig. 2). *Oobius agrili* positive trees appeared higher for yellow pan traps (64.0 ± 10.1%) and bark sifting (67.5 ± 9.8%) compared to bark rearing (22.3 ± 8.4%), although differences were not statistically significant (Fig. 2; F = 3.77; df = 2, 6; $P = 0.0923$).

3.2. Estimation of sample size

Bootstrapping results demonstrated that the probability of recovering *O. agrili* as a function of number of trees sampled varied among sampling methods and sites (Fig. 3; Supplemental Table A2A). When all trees within plots with yellow pan traps were bootstrap sampled, the probability function estimated that a sample size of 13 trees [confidence

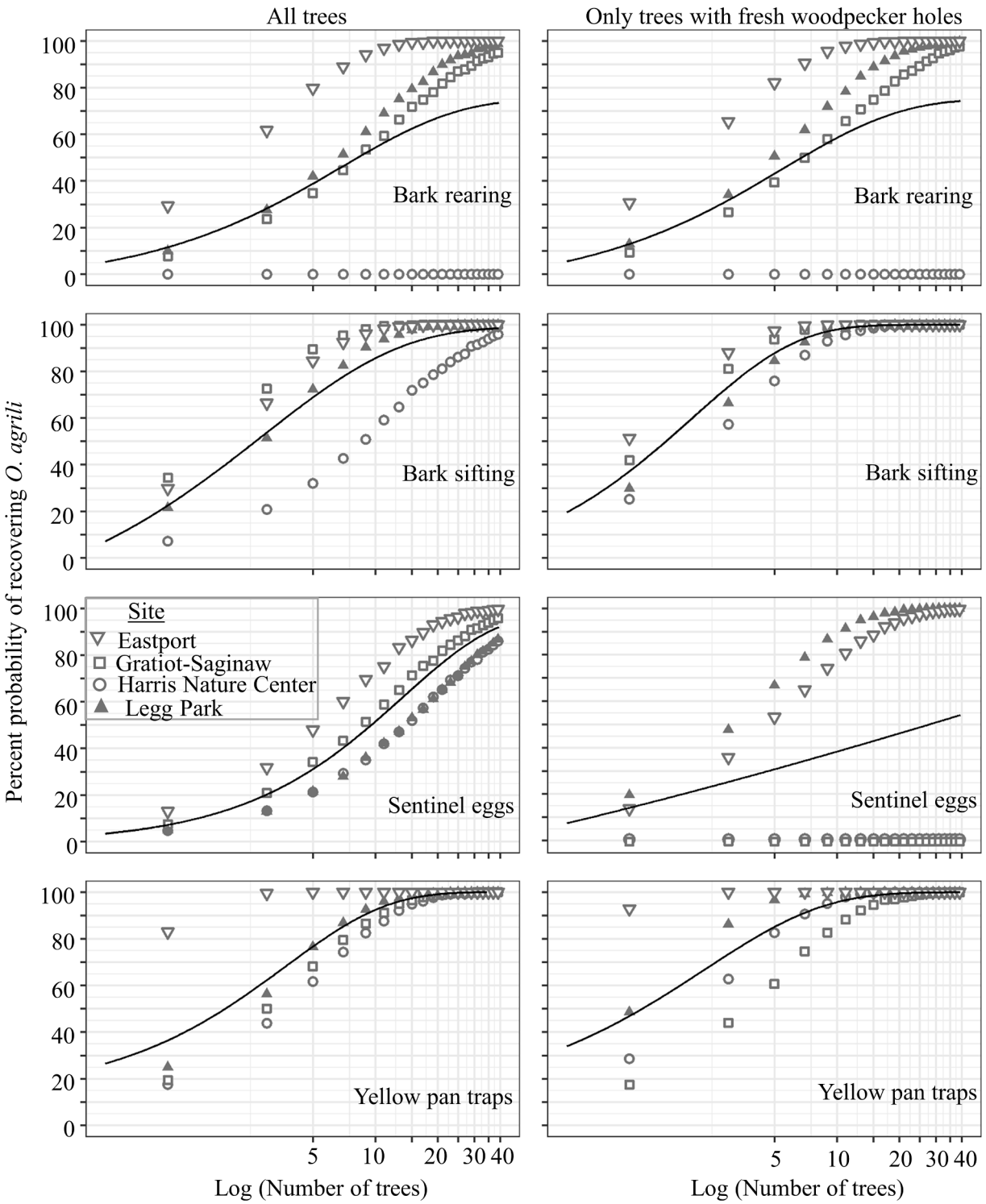

Fig. 3. Four-parameter Weibull probability function fitted to percentage of bootstrapping iterations with *O. agrili* recoveries for samples of 2–40 trees at four different sites [1) Eastport: Eastport, MI; 2) Gratiot-Saginaw: near Ithaca, MI; 3) Harris Nature Center and 4) Legg Park: Okemos, MI] for each of four sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps). Left column of figures includes all trees sampled at each site and right column includes only trees that had fresh woodpecker-feeding holes.

interval (CI) = 10–16] had a 95% probability of recovering *O. agrili*. Bark sifting required 16 trees (CI = 6–22) and sentinel eggs required 43 trees (CI = 27–70) to reach 95% probability of recovery when all trees were sampled. The probability of recovery never reached 95% for bark rearing due to failure to recover *O. agrili* at Harris Nature Center in 2016, where overall recoveries of *O. agrili* were very low (Table 2). Coefficients of variation for number of *O. agrili* recoveries when all trees within sites were randomly sampled were very high for all methods and sites (Range: 39.3–164.7% for sample size of 10 trees; Fig. 4). Coefficients of variation for the number of *O. agrili* recovered decreased as the number of trees sampled increased but the most dramatic decrease in coefficients of variation occurred when sample size increased from 2 to approximately 10 trees regardless of sampling method or site (Fig. 4).

Several signs and symptoms were significantly correlated with

Table 2
Correlation matrix showing p-value (*P*) and correlation coefficient (R) of the relationship between signs or symptoms of EAB attack (woodpecks = fresh woodpecker-feeding holes; epicormics shoots; exits = EAB exit holes; EAB eggs = total number of EAB eggs collected from bark samples) and recoveries of *O. agrili* adults or EAB eggs (parasitized and total) using four different sampling methods (bark rearing = bark rearing for adult *O. agrili*; bark sifting = bark sifting for parasitized EAB eggs; sentinel eggs = sentinel EAB egg screened envelopes; and yellow pan traps).

	Canopy dieback		Woodpecks pre-season		Epicormic shoots pre-season		Exits pre-season		Woodpecks post-season		Epicormic shoots post-season		Exits post-season		EAB eggs	
	P	R	*P*	R	*P*	R	*P*	R	*P*	R	*P*	R	*P*	R	*P*	R
Bark rearing recoveries	0.441	0.06	0.0008	0.26	0.011	0.20	0.855	0.01	<0.0001	0.40	0.298	0.08	<0.0001	0.30	<0.0001	0.40
Bark sifting recoveries	0.072	0.14	<0.0001	0.50	0.009	0.21	0.613	−0.04	<0.0001	0.46	0.635	0.04	<0.0001	0.50	<0.0001	0.74
Sentinel egg recoveries	0.719	0.03	0.0551	0.15	0.878	−0.01	0.133	0.12	0.011	0.20	0.287	−0.08	0.248	0.09	0.097	0.13
Yellow pan trap recoveries	0.357	0.07	<0.0001	0.67	0.438	0.06	0.527	−0.05	<0.0001	0.63	0.317	0.08	<0.0001	0.45	<0.0001	0.33
EAB eggs	<0.0001	0.33	0.0003	0.29	<0.0001	0.42	0.813	−0.02	<0.0001	0.35	0.931	0.01	<0.0001	0.40		

O. agrili recoveries for the different sampling methods (Supplemental Table A2). Abundace of fresh woodpecker-feeding holes was the most consistent sign or symptom correlated with *O. agrili* recoveries among sampling methods with correlations highly significant for bark rearing (fresh woodpecker-feeding holes post-season: $P < 0.0001$; $R = 0.40$), bark sifting (fresh woodpecker-feeding hole post-season: $P < 0.0001$; $R = 0.46$), and yellow pan traps (fresh woodpecker-feeding hole pre-season: $P < 0.0001$; $R = 0.67$), and marginally significant for sentinel eggs (fresh woodpecker-feeding hole pre-season: $P = 0.0551$; $R = 0.15$; Table 2). Therefore, bootstrap sampling was repeated using only trees with fresh woodpecker-feeding holes. Trees with pre-season fresh woodpecker-feeding holes in 2016 were used to subsample for yellow pan trap and sentinel egg methods because these were observed when yellow pan traps and sentinel eggs were first installed. Trees with post-season fresh woodpecker-feeding holes were subsampled for bark rearing and bark-sifting methods because these were observed in late-winter or early-spring 2017 when bark samples were collected. For all methods except for sentinel eggs, percentage probability of recovery increased with increasing number of sample trees that had fresh woodpecker-feeding holes compared to including all trees (Fig. 3; Supplemental Table A2). The number of trees with fresh woodpecker-feeding holes required to reach 95% probability of *O. agrili* recovery was 7 (CI = 6–8) and 10 (CI = 7–13) for bark sifting and yellow pan traps, respectively (Fig. 3). Of the three sites where *O. agrili* was recovered by bark rearing, the probability of recovery increased when sampling only trees with fresh woodpecker-feeding holes, but 95% was never reached because *O. agrili* was not recovered with bark rearing at Harris Nature Center in 2016 (Fig. 3; Table 1). For sentinel eggs, probability of recovery was lower when only trees with fresh woodpecker-feeding holes were sampled because no *O. agrili* were recovered on trees with fresh woodpecker-feeding holes at two of four sites (Fig. 3).

Coefficients of variation for *O. agrili* recoveries when only trees with fresh woodpecker-feeding holes were included were lower compared to those for all trees sampled, but still high, for all methods and sites (Range: 31.6–144.9% for sample size of 10 trees; Fig. 4). For sites and methods where *O. agrili* was recovered on trees with fresh woodpecker-feeding holes, coefficients of variation decreased dramatically as sample size increased to 10 trees and then decreased at a slower rate as sample size increased through 40 trees.

3.3. Sample timing

Mean GDD_{10} when yellow pan traps first collected *O. agrili* was 433 ± 25 (range: 342–625; Fig. 5). Mean peak *O. agrili* capture in yellow pan traps occurred at 845 ± 43 GDD_{10} (range: 617–1003; Fig. 5). Mean GDD_{10} when *O. agrili* collected reached 95% in yellow pan traps was 1068 ± 47 (range: 958–1407). Last capture of *O. agrili* in yellow pan traps was 1158 ± 56 GDD_{10} (range: 963–1407).

Mean GDD_{10} when parasitized sentinel eggs were first recovered was 774 ± 99 (range: 418–1185; Fig. 6). Mean GDD_{10} for peak sentinel egg parasitism was 891 ± 122 (range: 418–1199). Cumulative sentinel egg parasitism reached 95% at 984 ± 107 (range: 418–1199; Fig. 6).

4. Discussion

All four sampling methods recovered *O. agrili* in the field, including the three methods recommended by the EAB Biocontrol Guidelines (USDA–APHIS/ARS/FS, 2019). Of the methods tested, yellow pan traps and bark sifting recovered *O. agrili* in all sites and all years with consistently higher *O. agrili* recoveries and positive trees compared with bark rearing and sentinel eggs. Both yellow pan traps and bark sifting also had higher probability of recoveries with fewer sampled trees, compared to bark rearing and sentinel eggs. Sampling trees with fresh woodpecker-feeding holes increased the probability of recovering *O. agrili* for all methods except sentinel eggs and decreased the coefficients of variation for *O. agrili* recoveries. Each of these sampling

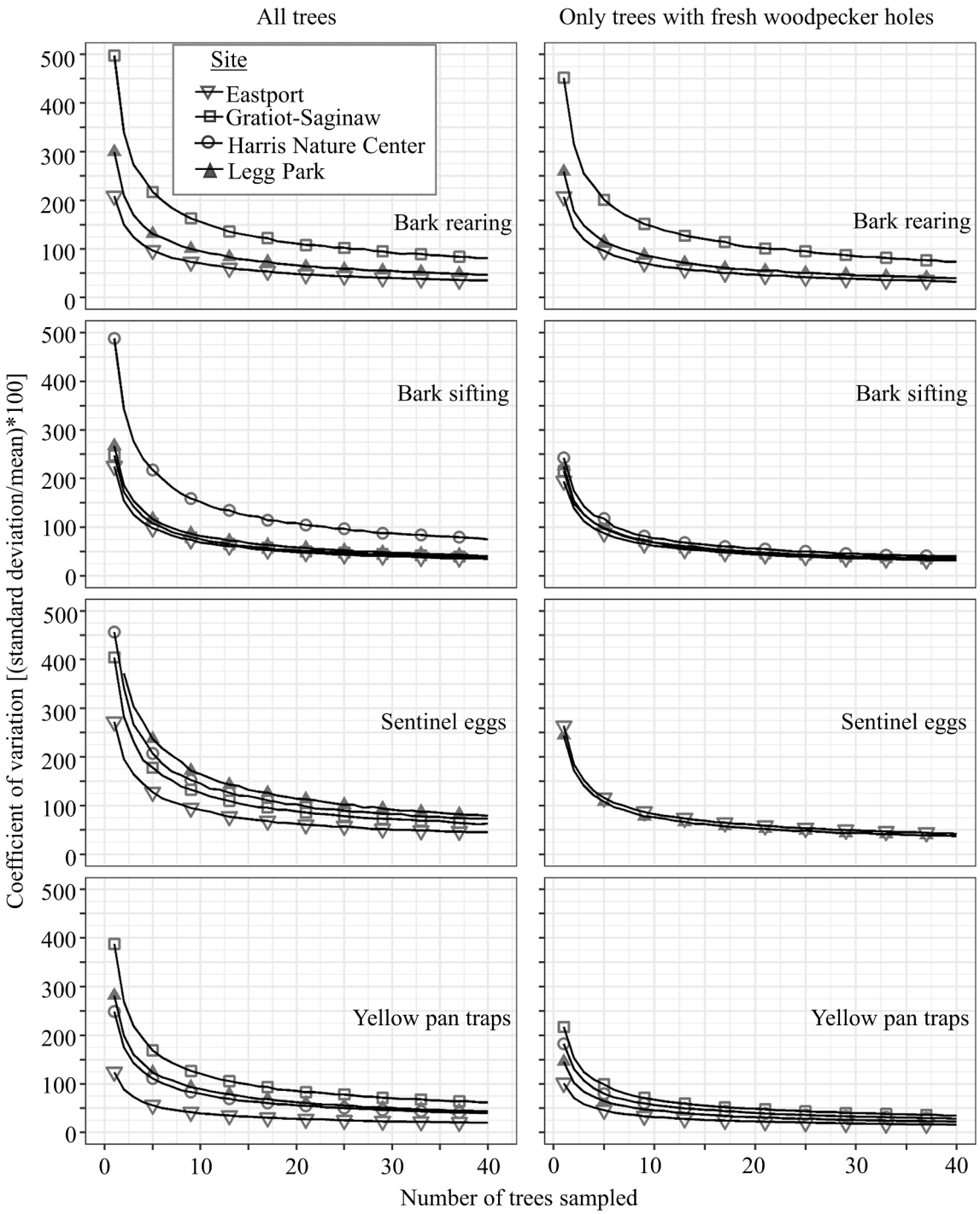

Fig. 4. Coefficient of variation of mean *O. agrili* recoveries for bootstrapped samples of 2–40 trees each iterated 2000 times for 4 sites in Michigan [1) Eastport: Eastport, MI; 2) Gratiot-Saginaw: near Ithaca, MI; 3) Harris Nature Center and 4) Legg Park: Okemos, MI] comparing 4 different sampling methods (bark rearing = bark rearing for adults; bark sifting = bark sifting for parasitized eggs; sentinel eggs = sentinel EAB eggs in screened envelopes; yellow pan traps). Left column of figures includes all 40 trees within sample plots at each site and right column includes only trees within each plot that had fresh woodpecker-feeding holes. Coefficients of variation are only shown for sites and methods that recovered *O. agrili* (see Fig. 3 and Supplemental Table A1).

methods has advantages and disadvantages, and each provides different information regarding *O. agrili* populations in the field (Table 3).

Capture of adult *O. agrili* in yellow pan traps confirms they are present at sites during the sampling season. Yellow pan traps also provide information on adult phenology, abundance, and seasonal activity, of *O. agrili* and other parasitoid species at release sites (Jones et al., 2019; Parisio et al., 2017). Disadvantages include assembly, because they are not commercially available, as well as installation and periodic collection when *O. agrili* adults are active, during spring and/or summer depending on geographic location. The EAB Biocontrol Guidelines (USDA–APHIS/ARS/FS, 2019) recommend sampling yellow pan traps every week using a solution of 25% propylene glycol and 75% water. Instead, we sampled every 2 weeks in 2016 using a solution of 50% propylene glycol and 50% water, and although the condition of samples was acceptable, samples collected weekly with a 50:50 solution during 2017 and 2018 were less decayed. Most *O. agrili* were captured in yellow

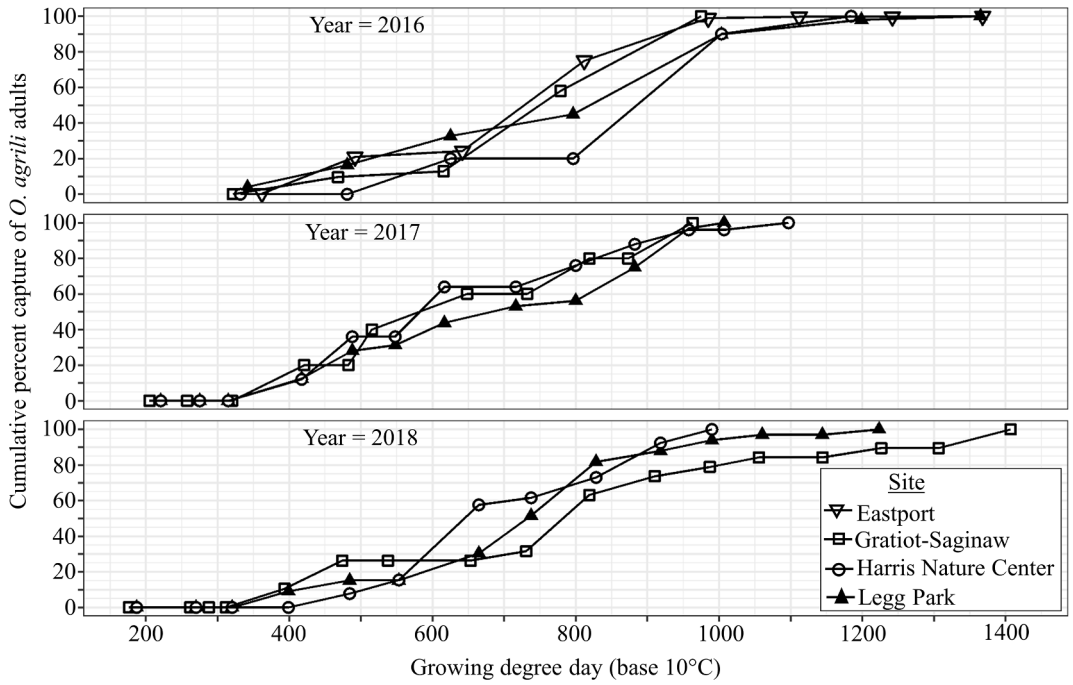

Fig. 5. Cumulative percentage capture of *O. agrili* adults in yellow pan traps by growing degree days (base = 10 °C; GDD_{10}) at four sites in Michigan (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) over a three-year period. Samples collected once every two weeks in 2016 and once every week in 2017 and 2018. Eastport was only sampled in 2016.

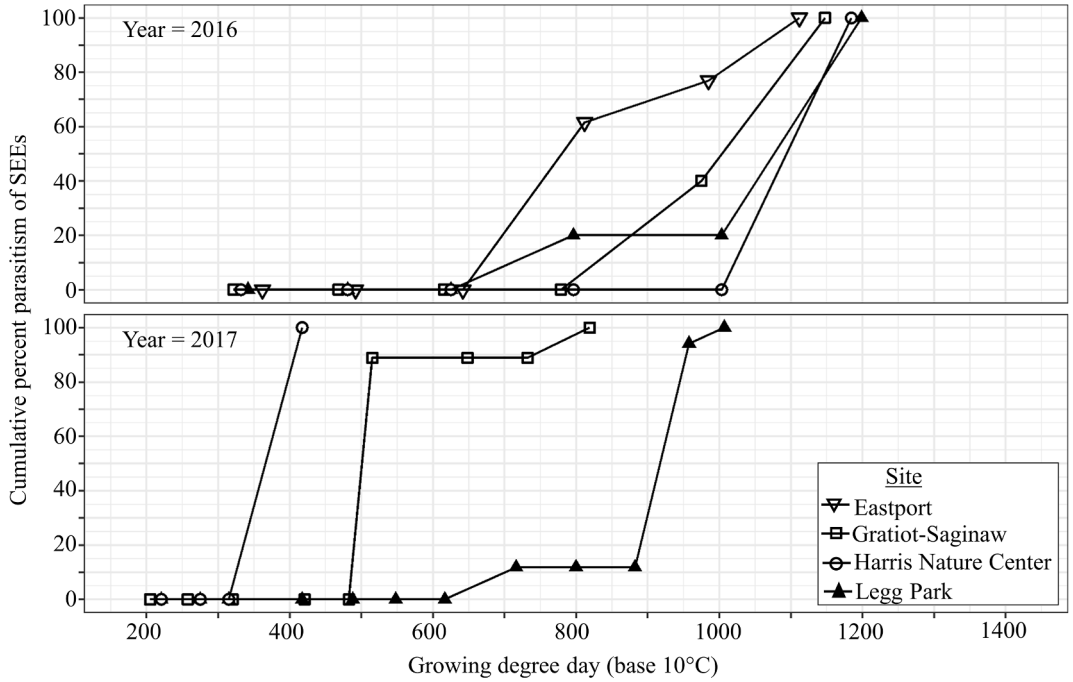

Fig. 6. Cumulative percentage parasitism of sentinel eggs by growing degree days (base = 10 °C; GDD_{10}) at four sites in Michigan (Eastport, MI; Gratiot-Saginaw, Ithaca, MI; Harris Nature Center, Okemos, MI; and Legg Park, Okemos, MI) over a two-year period. Samples were collected once every two weeks in 2016 and once every week in 2017. Eastport was only sampled in 2016.

pan traps between 433 and 1068 GDD_{10}, a mean trapping period of 54 ± 4 d (range = 41–85). Under Michigan climatic conditions, this length of trapping period for phenology studies would require approximately four collections at two-wk intervals or eight collections at one-wk intervals. After collection, sorting samples for suspect parasitoids can be labor intensive and challenging because *O. agrili* must be separated and distinguished from other insects, including several congeners.

The bark-sifting method is another effective *O. agrili* recovery method and can assess reproduction, establishment, and parasitism (Abell et al. 2014). A major advantage of this method is samples can be collected during a single site visit any time of the year. However, bark sifting samples are usually collected during fall through the following spring when *O. agrili* are in diapause. One caveat of bark sifting is that EAB eggs may persist on ash trees for more than one year, thus the

Table 3
For each of the four *O. agrili* sampling methods evaluated, we present an overview of the data each method can provide, as well as the number of site visits required, and the relative labor and technology required.

	Sampling methods			
	Bark rearing	Bark sifting	Sentinel eggs	Yellow pan traps
Confirms *O. agrili* currently present at site	Yes	No	Yes	Yes
Estimates percentage parasitism	No	Yes	Yes	No
Determines seasonal activity of adults	No	No	Yes	Yes
Collects other EAB parasitoids	No	No	No	Yes
Number of site visits	One	One	Several	Several
Technology required	Low	Low	High	Medium
Labor required	Low	Low	High	Medium

presence of EAB eggs with the signs and symptoms of previous *O. agrili* parasitism indicates they were present at some previous time since their release (Abell et al., 2014; Liu et al., 2007). Although both collecting and sifting bark samples take only a few minutes, examining bark debris under a dissecting microscope for EAB eggs can be tedious and requires approximately one hour per sample (Jennings et al., 2018; Pers. obs.), depending on the volume of bark debris.

Rearing *O. agrili* adults from bark samples was one of the least effective methods tested because it failed to recover *O. agrili* on two occasions where other methods successfully recovered *O. agrili*. For both of these occasions, overall *O. agrili* recoveries with other methods were low. Given this, bark rearing may not be effective for sampling areas where *O. agrili* densities are low. Also, the number of *O. agrili* reared from bark samples will never surpass parasitized eggs found during bark sifting because bark rearing is a subset of bark sifting, assuming that all parasitized eggs are recovered from bark sifting samples. An advantage of bark rearing is that bark-sifting samples can be reared prior to the bark-sifting process to quickly assess if *O. agrili* successfully overwintered at release sites. This requires bark samples be collected after *O. agrili* has experienced a period of cold temperatures (i.e., winter–-spring), allowing completion of diapause but before adult *O. agrili* emerge in the spring or summer (Duan and Larson, 2019; Petrice et al., 2019). After rearing is complete, bark can be sifted to examine for EAB egg parasitism. *Oobius agrili* recovery using the bark-rearing method can be improved by collecting bark from a larger surface area per tree and/or sampling from more trees. Alternatively, entire logs can be reared from felled ash trees, which Parisio et al. (2017) also found to be effective for recovering *O. agrili*.

Compared to the other methods evaluated in this study, the use of sentinel eggs was one of the least effective methods at recovering *O. agrili*, which is not surprising considering that *O. agrili* recovery success using sentinel eggs has varied among previous studies (Abell et al., 2006; Jennings et al., 2014a; Parisio et al., 2017). Parasitism of sentinel eggs was low despite the potential for volatiles from EAB eggs providing important host location cues for parasitoids (Vinson, 1998). However, volatiles produced by ovipositing EAB adults may provide additional host location cues (Colazza et al., 1999; Peri et al., 2006). In our study, we used EAB eggs laid on filter paper in the laboratory that would have little if any EAB adult volatiles present. It is important to note that the total number of *O. agrili* that could possibly be recovered in sentinel eggs in this study was limited to the number of EAB eggs placed on each tree (i.e., ten EAB eggs per tree). However, percent parasitism never reached 100% in any individual sentinel egg sample during our study, and therefore, we do not think this influenced the results. Despite the limitations of this method, sentinel eggs recovered *O. agrili* at all sites, including those with very low overall *O. agrili* recoveries for all methods, but mainly during the period of peak *O. agrili* adult densities (i.e. 418–1199 GDD_{10}). A major advantage of sentinel eggs is *O. agrili*'s presence can be confirmed if sentinel eggs are parasitized, which usually darken several days after attack (Abell et al., 2014), because *O. agrili* is the only egg parasitoid known to attack EAB in North America. Sentinel eggs can also provide data on both the seasonal activity and parasitism rates of *O. agrili* in the field (Abell et al., 2016; Duan et al., 2012). However, given the amount of labor and technology required for rearing EAB eggs, the multiple trips required for deployment and collection, and low overall recovery rates, the use of sentinel eggs is the least time- and cost-effective of the methods compared, especially if the primary objective is to determine *O. agrili* presence at release sites.

Sampling only trees with fresh woodpecker-feeding holes improved detection rates for most sampling methods. Woodpeckers primarily forage on late-instar EAB larvae, and fresh feeding holes confirm trees that were recently attacked if EAB is established in the area (Cappaert et al., 2005; Duan et al., 2010; Jennings et al., 2014b). The increase in probability of detecting *O. agrili* when sampling only trees with fresh woodpecker-feeding holes compared to sampling all trees was greater for bark sifting than for the other sampling methods. Since the bark-sifting method targets EAB eggs, it is intuitive that focusing on trees that were recently attacked by EAB improves detection because host eggs must be present for parasitized eggs to be recovered. Similarly, the probability of *O. agrili* detection using bark rearing increased when sampling ash trees with fresh woodpecker-feeding holes, compared to sampling all trees at the three sites where *O. agrili* was recovered by bark rearing (Fig. 3). Post-season EAB exit holes were also significantly correlated with *O. agrili* recoveries for bark sifting and bark rearing (Table 2). Including these trees may be considered when using these methods, but care must be taken to ensure that live phloem was available for EAB oviposition the previous season.

Sampling only trees with fresh woodpecker-feeding holes increased detection only slightly for yellow pan traps compared to sampling all trees. This was likely due to the high probability of detection when all trees were sampled, thus overshadowing any improvements in detection probabilities (Fig. 3). Yellow pan traps target *O. agrili* adults which can move freely throughout the environment in search of EAB eggs. Therefore, some *O. agrili* may be recovered on trees that were not recently attacked by EAB. Nevertheless, sampling trees with multiple fresh woodpecker-feeding holes should increase the number of *O. agrili* collected, given the relatively high correlation coefficient ($R = 0.6675$; Table 2) that described the relationship between fresh woodpecker-feeding holes and capture of *O. agrili* in yellow pan traps.

The probability of detecting *O. agrili* with sentinel eggs decreased when only trees with fresh woodpecker-feeding holes were sampled compared to sampling all trees because no *O. agrili* recovered from sentinel eggs on trees with fresh woodpecker-feeding holes at the two sites with the lowest overall number of fresh woodpecker-feeding holes. Selecting trees recently attacked by EAB may not be as important for the sentinel egg method compared to other methods because volatiles associated with the sentinel host eggs may attract *O. agrili* adults. However, the limited flight capacity of *O. agrili* would require sentinel eggs be deployed relatively close to trees where *O. agrili* adults are present (Parisio et al., 2017).

Regardless of the sampling method, coefficients of variation for *O. agrili* recoveries were moderate to very high. Some of this variation may be attributed to the inefficiency of the sampling methods used. Given the small size and limited flight capabilities of *O. agrili*, it is likely that *O. agrili* was present but not detected on some of the sampled trees at the study sites. For instance, *O. agrili* may have been present higher on tree trunks but not detected because sampling methods were applied to the lower tree trunk. Also, the tendency of *O. agrili* to be unevenly distributed within sites (Abell et al., 2016, 2014) likely contributed to the high variation in capture among trees. As expected, coefficients of variation decreased as the number of sample trees increased, improving *O. agrili* recovery precision. However, reductions in coefficients of variation were less dramatic after > 10 trees were sampled. Also,

coefficients of variation tended to be lower when trees with fresh woodpecker-feeding holes were sampled compared to sampling all trees within sites. Given these results, sampling a minimum of 10 trees with fresh woodpecker-feeding holes per site using bark sifting or yellow pan trap methods should provide a high confidence of *O. agrili* recovery if it is present at the site. This would also maximize the sample-size cost: benefit ratio for sampling precision. Much higher sample sizes may be needed for sentinel eggs and bark rearing methods to recover *O. agrili* if it is present.

CRediT authorship contribution statement

Toby R. Petrice: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Leah S. Bauer:** Conceptualization, Investigation, Methodology, Writing - review & editing. **Deborah L. Miller:** Conceptualization, Investigation, Methodology, Writing - review & editing. **John S. Stanovick:** Conceptualization, Writing - review & editing. **Therese M. Poland:** Conceptualization, Resources, Funding acquisition, Project administration, Writing - original draft, Writing - review & editing. **F. William Ravlin:** Conceptualization, Resources, Funding Administration, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2021.104535.

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