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NOTE

Evaluating methods to detect and monitor North American larval parasitoids of the emerald ash borer (Coleoptera: Buprestidae)

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Abstract
Populations of native North American parasitoids attacking *Agrilus* Curtis (Coleoptera: Buprestidae) species have recently been considered as part of an augmentative biological control programme in an attempt to manage emerald ash borer, *Agrilus planipennis* Fairmaire, a destructive wood-boring beetle discovered in North America in 2002. We evaluate trapping methods to detect and monitor populations of two important native larval parasitoids, *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) and *Atanycolus* Förster (Hymenoptera: Braconidae) species, attacking emerald ash borer in its introduced range. We found that purple prism traps captured more *P. sulcata* than green prism traps, yellow pan traps, and log samples and thus were considered better for detecting and monitoring *P. sulcata* populations. Trap type did not affect the number of captures of *Atanycolus* species. Surprisingly, baiting prism traps with a green leaf volatile or manuka oil did not significantly increase captures of *P. sulcata* or *Atanycolus* species. Based on these results, unbaited purple prism traps would be optimal for sampling these native emerald ash borer parasitoids in long-term management programmes.

There are a number of native parasitoids that attack native *Agrilus* Curtis (Coleoptera: Buprestidae) in North America and have been observed to also attack the introduced emerald ash borer, *Agrilus plannipennis* Fairmaire (Coleoptera: Buprestidae), a major forest pest in Canada and the United States of America. Specifically, native larval parasitoids *Phasgonophora sulcata* Westwood (Hymenoptera: Chalcididae) and *Atanycolus* Förster (Hymenoptera: Braconidae) species are important mortality factors of emerald ash borers in Canada. Emerald ash borer parasitism has been observed as high as 40% by *P. sulcata* and 71% by *Atanycolus cappaerti* Marsh and Strazanac in some sites (Cappaert and McCullough 2009; Lyons 2010). It appears that these parasitoids have a strong, localised impact on emerald ash borer populations (Gaudon 2019), which may be partially explained by weak dispersal (Gaudon *et al.* 2018) and ecosystem characteristics such as tree species and tree condition (Gaudon 2019).

Several emerald ash borer parasitoids, including species from Asia and North America, have been identified for biological control programmes to manage emerald ash borer populations

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(Bauer *et al.* 2015; Roscoe 2014; Gaudon 2019), and recent research has incorporated native emerald ash borer parasitoids as part of a long-term management effort (Gaudon and Smith 2019). To determine the success of such trials, sampling tools are needed to confirm the establishment of translocated parasitoids and monitor their populations over time. The ability to detect and monitor these natural enemies is critical if they are to be used successfully for biological control, especially in terms of their establishment and impact (Van Driesche and Bellow 1996). Moreover, the variation in native emerald ash borer parasitoid abundance across eastern North America necessitates the development of an efficient trap for their sampling (*i.e.*, one that, at minimum, could detect the presence or absence of native emerald ash borer parasitoids). In general, sampling for native emerald ash borer parasitoids is labour intensive and involves the cutting and sampling of large logs. This approach is destructive and time-consuming and requires considerable resources. Thus, there is a need for a less destructive and cost-effective sampling technique to survey and monitor the establishment of native emerald ash borer parasitoids before augmentative releases to facilitate the identification of high-priority release sites.

Adult parasitoids can be sampled using a wide variety of techniques (*e.g.*, Malaise traps, yellow pan traps, sweep netting), and different sampling methods will collect different groups of parasitoids (Price 1971; Darling and Packer 1988; Aguiar and Santos 2010; McCravy 2018). While some of these recovery techniques allow for monitoring the establishment and impact of parasitoid populations in the emerald ash borer system (*e.g.*, Duan *et al.* 2013; Hooie *et al.* 2015; Jennings *et al.* 2018), they have only focussed on methods to survey introduced Asian emerald ash borer parasitoids (Parisio *et al.* 2017) and not native parasitoids.

Traps designed to capture both host insects and their parasitoids would be extremely useful (*e.g.*, Derocles *et al.* 2014) and appear promising for emerald ash borer and its parasitoids (Roscoe 2014). A recent meta-analysis of trap designs used in monitoring Buprestidae found that panel traps captured more than multiple-funnel traps, while traps treated with a slippery coating (*e.g.*, Fluon or Teflon) increased captures compared to those left untreated (Allison and Redak 2017). Allison and Redak (2017) also found that black traps were better at capturing insects compared to white or clear traps and that purple traps were equal or superior to green traps, especially for capturing *Agrilus*. It appears then that colour acts as an important visual stimulus for *Agrilus* beetles and this may also be true for shape as the tree-like appearance of prism traps may provide an attractive visual silhouette as seen with Lindgren-funnel traps for Scolytinae (Coleoptera: Curculionidae) (Lindgren 1983; Chénier and Philogène 1989). Currently, attractant-based traps are considered the most effective for detecting low-density populations of Buprestidae; however, we do not know whether any of the traps now used to sample emerald ash borers (*e.g.*, baited purple or green prism traps) can also be used to monitor native emerald ash borer parasitoids.

Here, we evaluated the efficacy of trap type and bait to identify the optimal method for monitoring native North American emerald ash borer parasitoids and emerald ash borers following future releases in augmentative biological control. Specifically, our objective was to compare trap type and bait to assess what captured greater numbers of native emerald ash borer parasitoids. We predicted that dark-coloured, prism-shaped traps (purple prism) treated with a bait (host plant and/or host beetle chemical) would capture the greatest number of native emerald ash borer parasitoids on the basis that a parasitoid of Buprestidae would use similar visual signals to orient as their host.

Two experimental field trials were used to test different trap designs over three nonconsecutive years. In the first trial (McKeough Floodway in Wilkesport, Ontario, Canada), comparisons were made between trap type and bait using six purple prism traps, six green prism traps, and 30 sticky band traps; half of the purple prism traps ($n = 3$) were baited with manuka oil and half of the green prism traps ($n = 3$) were baited with a green leaf volatile, (Z)-3-hexenol. Both volatiles have been used for capturing emerald ash borers (*e.g.*, Crook *et al.* 2012), and Roscoe (2014) found that *P. sulcata* were attracted to the green leaf volatile in behavioural assays and that it elicited a weak antennal response from *P. sulcata* in the laboratory. Prism traps were hung in the canopy of ash trees (*Fraxinus* Linnaeus; Oleaceae), while sticky band traps were deployed on the trunk at breast height, that is, approximately 1.3 m high. The traps were deployed between 2–4 June 2010, and *P. sulcata*, *Atanycolus* species, and emerald ash borers were collected from them approximately every two weeks until 9–12 September 2010; specimens were brought to the Great Lakes Forestry Centre (Sault Ste. Marie, Ontario, Canada) for identification.

Yellow pan traps are commonly used to sample introduced emerald ash borer parasitoids (United States Department of Agriculture 2015). Trial 2 compared *P. sulcata* and *Atanycolus* captures between 15 yellow pan traps, 15 sticky band traps, and 15 purple prism traps. Yellow pan traps were similar to those used by the United States Department of Agriculture (2015) and positioned on the southwest side of ash trees. Prism traps were purchased from Synergy Semiochemicals Corporation (Delta, British Columbia, Canada), and sticky band traps were made from approximately 51-cm-wide plastic wrap (item number 498385, Staples Canada: www.staples.ca) coated with Pestick insect glue (catalogue number 01-3522-2, Hummert International: www.hummert.com). Traps were deployed on ash trees on 25 May 2016 at a second study site in a naturalised area (Merchants Trail in Oakville, Ontario). *Phasgonophora sulcata*, *Atanycolus*, and emerald ash borers were collected from the yellow pan traps every week and from the sticky band and purple prism traps every two weeks until 18 August 2016. The following year (2017), the same number of yellow pan traps, sticky band traps, and purple prism traps were installed on ash trees and collected as in 2016 to examine variability between years. Parasitoids and emerald ash borers were also sampled at this site during 30 April 2017 by cutting three approximately 60-cm logs from 12 living ash trees ($n = 36$) in order to rear *P. sulcata, Atanycolus*, and emerald ash borers and compare the number of parasitoids to those caught in the pan, sticky, and prism traps. The expectation was that the logs would provide a baseline estimate of parasitoid and emerald ash borer density at that site. Ash trees typically showed signs and symptoms of emerald ash borer infestation, including canopy decline, bark splits, D-shaped exit holes, and woodpecker feeding, and were 11.7 ± 0.4 cm (mean ± standard error) diameter at breast height.

The total numbers of parasitoids and emerald ash borers caught per trap were summed over the total sampling period and used in the analysis. The *Atanycolus* species discussed herein includes *A. cappaerti*, *A. hicoriae* Shenefelt, *A. disputabillis* Cresson, *A. tranquebaricae* Shenefelt, and *A. longicauda* Kokujev, with *A. cappaerti* being the dominant species captured. Insects were identified using keys (Paiero *et al.* 2012; Roscoe 2014) and by specialists (D.B. Lyons, J.M. Gaudon, P. Marsh, and L. Roscoe). Voucher specimens were deposited at the Great Lakes Forestry Centre (Sault Ste. Marie, Ontario, Canada) and University of Toronto (Toronto, Ontario, Canada). Data from the two trials were analysed separately.

All data were analysed in R (R Development Core Team 2018). Generalised linear models with Poisson errors and log link functions were fit to test the effect of trap type on insect counts in Trial 1 and the effects of trap type and year on insect counts in Trial 2. Each model fit was examined by comparing the residual deviance and degrees of freedom to look for overdispersion or underdispersion. These models did not meet our assumption of overdispersion, so negative binomial models that accounted for the overdispersion in our data were used instead. We fit negative binomial models with functions in the package "MASS," and *post hoc* tests were performed using functions in the package "agricolae." All interaction terms were initially considered, but in cases where models would not converge if interactions were included, only main effects were explored. In all other cases, nonsignificant interaction terms were sequentially removed from the models to, at minimum, explore main effects (*i.e.*, trap type and year). Tukey's range test was used to explore significant differences between main effects at alpha $= 0.05$.

A total of 1618 *P. sulcata*, 129 *Atanycolus*, and 1434 emerald ash borers were captured in Trial 1. A maximum of 191 *P. sulcata* were captured on one baited purple prism trap, and no *P. sulcata* were collected on baited green prism traps in the same trial. Fewer emerald ash borers and *Atanycolus* were captured than *P. sulcata* at this site, with a maximum number of 91 emerald ash borers found on one unbaited purple prism trap and 22 *Atanycolus* found on one sticky band trap in 2010. Trap type significantly affected the number of *P. sulcata* captured ($\chi^2 = 47.60$, df = 4, $P < 0.001$) in Trial 1. Unbaited purple prism traps captured significantly more *P. sulcata* than baited purple prism traps, baited purple prism traps captured significantly more *P. sulcata* than sticky band traps, sticky band traps captured significantly more *P. sulcata* than unbaited green prism traps, and unbaited green prism traps captured significantly more *P. sulcata* than baited green prism traps (Table 1). The number of emerald ash borers captured was also significantly influenced by trap type ($\chi^2 = 19.29$, df = 4, $P = 0.001$), where more emerald ash borers were found on baited green prism traps than those left unbaited, and more emerald ash borers were found on unbaited green prism traps than both baited and unbaited purple prism traps. Fewer emerald ash borers were captured on sticky band traps than all other trap types (Table 1). The number of *Atanycolus* captured was not influenced by trap type ($\chi^2 = 7.50$, df = 4, $P = 0.112$).

Fewer parasitoids and emerald ash borer overall were captured in Trial 2 than Trial 1. A total of 77 *P. sulcata*, 38 *Atanycolus*, and 152 emerald ash borers were captured in 2016 and 29 *P. sulcata*, three *Atanycolus*, and 143 emerald ash borers in 2017. A maximum number of 21 *P. sulcata* were collected on a purple prism trap in 2016 versus 10 *Atanycolus* on one sticky band trap in 2016. More emerald ash borers were captured than parasitoids, with a maximum of 39 emerald ash borers captured on one purple prism trap in 2016. The number of *P. sulcata* captured was significantly influenced by trap type ($\chi^2 = 90.02$, df = 3, $P < 0.001$) and year ($\chi^2 = 4.18$, df = 1, $P = 0.041$) in Trial 2. Significantly greater numbers of *P. sulcata* were captured using purple prism traps than all other trap types (sticky band traps and yellow pan traps) or by taking log samples (Table 2). Captures of *P. sulcata* were significantly higher in 2016 than 2017 (Table 2). In Trial 2, the number of *Atanycolus* captured was not influenced by trap type ($\chi^2 = 6.23$, df = 3, $P = 0.101$); however, year did significantly affect the number of *Atanycolus* collected ($\chi^2 = 21.55$, df = 1, $P < 0.001$). Similar to *P. sulcata*, more *Atanycolus* were captured in 2016 than 2017 (Table 2). The number of emerald ash borer captured was significantly influenced by trap type ($\chi^2 = 132.08$, df = 3, $P < 0.001$), with greater numbers of emerald ash borer captured on purple prism traps than sticky band traps (Table 2). Not surprisingly, greater numbers of emerald ash borer were captured on sticky band traps than both yellow pan traps and logs (Table 2). Year did not significantly affect the number of emerald ash borer collected ($\chi^2 = 0.04$, df = 1, $P = 0.848$).

Purple prism traps were better at capturing *P. sulcata* than green prism traps, sticky band traps, or yellow pan traps under similar conditions. In contrast, trap type did not influence the total number of *Atanycolus* collected. Prism traps captured more emerald ash borer than sticky bands or yellow pan traps, with baited and unbaited green prism traps capturing greater numbers than baited or unbaited purple prism traps. Studies that investigate traps that can be used to sample both a parasitoid and its host are rare (although see Derocles *et al.* 2014), but our work shows that emerald ash borer and its native parasitoids can be sampled in the field using only one trap type (*i.e.*, purple prism traps, although emerald ash borer captures on purple prism traps were lower than green prism traps) and that this may provide a particularly cost-effective tool for targeting future biological control programmes with native parasitoids against emerald ash borers.

It would be ideal if purple prism traps could also sample recently introduced emerald ash borer parasitoids such as *Tetrastichus planipennisi* Yang (Hymenoptera: Eulophidae), *Oobius agrili* Zhang and Huang (Hymenoptera: Encyrtidae), and *Spathius agrili* Yang (Hymenoptera: Braconidae). Parisio *et al.* (2017) found that yellow pan traps were comparable to sentinel logs and girdled trap trees in terms of their ability to detect the presence of introduced emerald ash borer parasitoids; however, we found that yellow pan traps took more time to install and service than other traps and did not recover native *P. sulcata*. Thus, it may be that multiple trap types are needed to sample for both introduced and native emerald ash borer parasitoids in North America.

Table 1. Minimum, maximum, and mean (± 1 standard error (SE)) number of *Phasgonophora sulcata*, *Atanycolus*, and emerald ash borer captured on unbaited purple prism, baited purple prism, sticky band, unbaited green prism, and baited green prism traps in the McKeough Floodway (Wilkesport, Ontario, Canada) in 2010 (Trial 1). Purple prism traps were baited with manuka oil, and green prism traps were baited with the green leaf volatile (Z)-3-hexenol.

		Phasgonophora sulcata			*Atanycolus*			Emerald ash borer		
Trap type	Number of traps	Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE
Purple prism – unbaited	3	48	123	76 ± 24[a]	1	11	5 ± 3	39	91	58 ± 16[a]
Purple prism – baited	3	9	191	73 ± 59[b]	0	6	4 ± 2	40	74	58 ± 10[a]
Sticky band	30	2	127	39 ± 6[c]	0	22	3 ± 1	2	88	23 ± 4[b]
Green prism – unbaited	3	1	5	3 ± 1[d]	0	1	0 ± 0	54	73	64 ± 6[c]
Green prism – baited	3	0	0	0 ± 0[e]	0	1	1 ± 0	60	80	71 ± 6[d]

Note: Significant differences at $P < 0.05$ between trap type using Tukey's range test are indicated by different lowercase letters.

Table 2. Minimum, maximum, and mean (± 1 standard error (SE)) number of *Phasgonophora sulcata*, *Atanycolus*, and emerald ash borer captured on unbaited purple prism, sticky band, and yellow pan traps, and found emerging from logs in a naturalised area in Oakville, Ontario, Canada (Merchants Trail) in 2016 and 2017 (Trial 2).

			Phasgonophora sulcata			*Atanycolus*			Emerald ash borer		
Year	Trap type	Number of traps	Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE	Minimum	Maximum	Mean ± SE
2016	Purple prism – unbaited	15	0	21	6 ± 2[a]	0	5	1 ± 0	0	39	12 ± 3[a]
	Sticky band	15	0	0	0 ± 0[b]	0	10	1 ± 1	0	2	0 ± 0[b]
	Yellow pan	15	0	0	0 ± 0[b]	0	5	0 ± 0	0	0	0 ± 0[c]
	Log sample	0	–	–	–[b]	–	–	–	–	–	–[c]
2017	Purple prism – unbaited	15	0	15	2 ± 1[a]	0	1	0 ± 0	0	23	8 ± 2[a]
	Sticky band	15	0	0	0 ± 0[b]	0	1	0 ± 0	0	16	1 ± 1[b]
	Yellow pan	15	0	0	0 ± 0[b]	0	0	0 ± 0	0	0	0 ± 0[c]
	Log sample	36	0	0	0 ± 0[b]	0	0	0 ± 0	0	0	0 ± 0[c]

Note: Significant differences at $P < 0.05$ between trap type using Tukey's range test are indicated by different lowercase letters.

Annual variation in the population sizes of both *P. sulcata* and *Atanycolus* will affect the total number of adults caught irrespective of trap type, and thus one must consider the age of the emerald ash borer infestation when sampling for these parasitoids. No parasitoids emerged from logs cut at the Oakville site in Trial 2 (Table 2), and this may be due to the age of the emerald ash borer infestation at that site and explain the differences observed between sampling years (2016 and 2017). Several mature ash trees at the site were dead, while the young ash trees showed fewer signs and symptoms of emerald ash borer suggesting that the infestation was older and had lower populations of emerald ash borer and parasitoids. Similarly, no emerald ash borer emerged from cut logs. Burr *et al.* (2018) showed that sites with newer or older emerald ash borer infestations had lower emerald ash borer populations relative to "crest sites" with peak infestations, and thus we speculate that this may also be true for native parasitoids attacking emerald ash borer. Higher parasitism has been observed on stressed trees compared with relatively healthy trees for cerambycid beetles (Coleoptera: Cerambycidae) (Shibata 2000; Flaherty *et al.* 2011), and it is possible that native emerald ash borer parasitoids follow a similar foraging pattern.

Trap placement and feeding guild are important considerations when monitoring insect populations. Ulyshen and Sheehan (2019) found greater numbers of phloem and wood-boring beetles in traps high above the ground, while more ambrosia beetles were found in traps near the ground. Similarly, Allison *et al.* (2019) reported significant effects on the capture of adult cerambycids for traps placed along transects perpendicular to forest edges. In our study, it appears that traps placed in tree canopies (prism traps) captured more emerald ash borer parasitoids than those placed on the main trunk (sticky bands and yellow pan traps). This is not surprising as prism traps also captured more emerald ash borers than sticky bands and yellow pan traps, which might suggest that the emerald ash borer infestation within our study sites occurred in the canopy. As such, survey programmes should consider placing traps near actively infested locations in order to effectively detect native emerald ash borer parasitoids and emerald ash borer.

We found that baiting prism traps with manuka oil or a green leaf volatile did not significantly increase parasitoid captures compared to unbaited prism traps. Moreover, our trap captures in the field of both *P. sulcata* and *Atanycolus* were either the same or less with the addition of a green leaf volatile – (Z)-3-hexenol – bait, despite *P. sulcata* showing a behavioural response to the same green leaf volatile in the laboratory (Roscoe 2014). It is possible that these parasitoids use a combination of semiochemicals to locate emerald ash borer hosts, including compounds from the host itself (*e.g.*, 3-(Z)-lactone, a pheromone released by emerald ash borer when it feeds on ash foliage (Bartelt *et al.* 2007)) under field conditions, and these were lacking in laboratory studies and the traps we had deployed.

Trap colour and placement appear to be more important than trap surface area for those parasitoid species studied here. Although the surface area of the purple prism traps used in our work was greater than that of the yellow pan traps, it was similar to that of the green prism traps and some sticky band traps, both of which captured significantly fewer *P. sulcata* than the purple prism traps. Furthermore, the number of *Atanycolus* collected was similar across all trap types regardless of their surface area. It is possible that multiple factors work synergistically in the field to determine the efficacy of traps for native emerald ash borer parasitoids. For example, more *P. sulcata* might be captured on purple prism traps in the canopy than on the lower bole of trees, especially in early infestations as emerald ash borer is thought to typically infest the upper canopy of large trees first (Cappaert *et al.* 2005). Moving forward, future studies should investigate interactions between different trap colours and placement in the tree and stand, along with alternative baits (such as 3-(Z)-lactone, or a combination of baits such as 3-(Z)-lactone plus a green leaf volatile) to improve catches of native parasitoids in future biological control programmes against emerald ash borer.

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