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Interactions between *Spathius agrili* (Hymenoptera: Braconidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae), larval parasitoids of *Agrilus planipennis* (Coleoptera: Buprestidae)

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ABSTRACT

Three hymenopteran parasitoids native to China are being released in the United States as biological control agents for the emerald ash borer (EAB), *Agrilus planipennis* Fairmaire, an Asian buprestid species responsible for mortality of ash trees (*Fraxinus* spp.) in North America. Two of these hymenopterans, *Spathius agrili* Yang (Braconidae), a larval ectoparasitoid, and *Tetrastichus planipennisi* Yang (Eulophidae), a larval endoparasitoid, prefer late-instar EAB larvae. This overlapping host preference raises concerns that interspecific competition following field releases may compromise establishment of one or both species. In a series of laboratory and field experiments, we found *S. agrili* and *T. planipennisi* exhibited similar parasitism rates when presented alone with EAB larvae for 12–14 days. However, *S. agrili* was more efficient at locating and parasitizing hosts within the first 27 h, possibly explaining why *S. agrili* excluded *T. planipennisi* in the laboratory trials and nearly excluded *T. planipennisi* in field trials when the two species were presented together with EAB larvae. We found that *S. agrili* parasitized larvae previously parasitized by *T. planipennisi* but not the reverse. However, *S. agrili* offspring failed to complete development on hosts that were previously parasitized by *T. planipennisi*. We recommend releasing these species separately in time or space to avoid the antagonistic interactions observed in this study.

1. Introduction

Since first detected in Michigan and Ontario in 2002 (Haack et al., 2002), the emerald ash borer (EAB) (*Agrilus planipennis* Fairmaire), a buprestid native to Asia, has killed tens of millions of ash trees (*Fraxinus* spp.) in North America and continues to expand into new areas. Three species of hymenopteran parasitoids associated with EAB in China are being introduced into Michigan and other EAB-infested states in an effort to manage this destructive beetle using biological control (Bauer et al., 2008).

Whether to introduce a single or multiple species in biological control remains an active area of debate (Mills, 2006). However, a consistent conclusion reached in reviews of multiple-agent biological control projects targeting insect pests is that establishment rates decrease with increasing numbers of introductions (Ehler and Hall, 1982; Denoth et al., 2002). Moreover, multiple-agent biological control projects have been shown to be no more successful than single-agent projects (Denoth et al., 2002). As these trends have been attributed to competitive exclusion (Ehler and Hall, 1982; Denoth et al., 2002), it is important to understand what kinds of interactions to expect between biological control agents intended for release.

The three species of parasitic wasp being released to control EAB are *Oobius agrili* Zhang and Huang (Encyrtidae), an egg parasitoid, and *Spathius agrili* Yang (Braconidae) and *Tetrastichus planipennisi* Yang (Eulophidae), both larval parasitoids. As an egg parasitoid, *O. agrili* will clearly not compete directly with *S. agrili* or *T. planipennisi*. However, there may be considerable potential for interspecific competition between the two larval parasitoids, *S. agrili* and *T. planipennisi*.

Spathius agrili is a gregarious idiobiont ectoparasitoid of late-instar EAB larvae (Yang et al., 2005). Adults range from 3.4 to 4.3 mm in length with a female to male sex ratio of ~3:1 (Yang et al., 2005). Females permanently paralyze their hosts by envenomation during ovipositioning and produce 1–18 offspring per host (Yang et al., 2005). In China, they complete up to four generations a year and levels of parasitism range from 30% to 90% (Yang et al., 2005).

Tetrastichus planipennisi is a gregarious koinobiont endoparasitoid of late-instar EAB larvae. Adults range from 1.6 to 4.1 mm in length with a female to male sex ratio of ~2.5:1 (Yang et al., 2006). Larvae parasitized by *T. planipennisi* remain active and con-

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tinue to feed for about a week (J.J.D., unpublished data). Between 4 and 172 offspring are produced per host (M.D.U., unpublished data). After consuming the host larva, parasitoid larvae exit from the integument and pupate within the EAB gallery (Yang et al., 2006). Adult wasps eclose approximately 15 days after pupation and exit the tree through one or more holes chewed through the bark (Yang et al., 2006). In China, four or more generations are produced each year and levels of parasitism average 22.4% (Liu et al., 2007), ranging from 0% to 65% (Liu et al., 2003, 2007; Yang et al., 2006).

The extent to which antagonistic interactions between *S. agrili* and *T. planipennisi* may compromise establishment of one or both species at release sites remains unknown. The purpose of this project was to determine which species, if either, has the competitive advantage and why. The project consisted of three parts. We first carried out a series of dominance assays to determine which species has the competitive advantage. We then performed a comparison of short-term parasitism rates to determine which species was more efficient at locating hosts. Finally, we carried out multiparasitism assays in which we tested whether hosts parasitized by one species were acceptable to the other. The first two parts of the project focused on competition among adult wasps searching for hosts. The third part examined the extent to which competition is likely to occur among larvae.

2. Materials and methods

2.1. Parasitoids

Laboratory cultures of *T. planipennisi* and *S. agrili* were used in this study. Only naïve wasps that had never been presented with hosts were used in experiments. They were generally over a week old at the time of use and were presumed to have mated based on the observation that mating occurs rapidly for both species in the laboratory. Only females were used in experiments, unless otherwise stated for *T. planipennisi* (however, the presence of males does not appear to affect parasitism rates, M.D.U., pers. obs.). The ratios of *T. planipennisi* and *S. agrili* used in this study (5:2 or 10:3) were chosen based on the preliminary observation that *S. agrili* was more efficient at locating hosts (i.e., in order to avoid complete dominance by *S. agrili*). The ratios of the two species occurring in nature are not known. The numbers of *T. planipennisi* and *S. agrili* used per replicate in each experiment were held constant to control for intraspecific competition. The extent to which intraspecific competition takes place for either species is not well understood.

2.2. Dominance assays

The extent to which *T. planipennisi* or *S. agrili* dominates was assessed by comparing the following treatments: (1) EAB larvae presented to *T. planipennisi* only, (2) EAB larvae presented to *S. agrili* only and (3) EAB larvae presented to *T. planipennisi* and *S. agrili* simultaneously. This experiment was carried out both in the laboratory and in the field, as described below and outlined in Table 1.

2.2.1. Laboratory trials

Five ♀ *T. planipennisi* and two ♀ *S. agrili* were presented alone and together with 4th instar field-collected EAB larvae inserted into ~10 cm long ash (*Fraxinus* spp.) sticks. The experiment was performed twice, once with ~1 cm diameter sticks and again with ~4.5 cm diameter sticks (hereafter referred to as "small" or "large" sticks, respectively). The sticks were collected from healthy trees in the field. To reduce fungal growth, the sticks were gently scrubbed under running tap water, sealed at both ends with paraffin, held in a 0.05% bleach bath for ca. 5 min, and rinsed with running tap water for 15 min. Fourth instar larvae were inserted into narrow grooves chiseled beneath small flaps of bark peeled from one end of each stick. Upon insertion, the head of each larva was aimed away from the insertion end of the stick to encourage feeding along the length of the stick. The bark flaps were then closed over the inserted larvae and held closed with thin strips of Parafilm. One EAB larva was inserted into each small stick and five larvae were spaced equally around one end of each large stick. Five small sticks and one large stick (i.e., five larvae in each case) were placed in 473 and 710 ml clear plastic drinking cups (GFS.com), respectively, with the ends containing the larvae facing up (i.e., with larvae feeding down the length of the sticks). The small sticks were placed in the cups side-by-side in alternating horizontal directions (Fig. 1A) whereas the large sticks were held upright using a tack punched through the bottom of each cup (Fig. 1B). The cup openings were covered by fine screen held in place by lids in which 5.8 cm diameter circular openings had been made. Wasps were then added to each cup. The cups were held in an incubator (25 °C, 16:8 light:-dark, ~75% humidity) with food (drops of honey) and water (moistened cotton balls) added to the tops of the screens daily. After 12 days, the sticks were dissected and the EAB larvae were placed individually in Petri dishes lined with moistened filter paper. EAB exposed to *S. agrili* alone or in combination with *T. planipennisi* were immediately examined externally for *S. agrili* larvae and/or eggs. For EAB larvae exposed to both species, the remains of larvae parasitized by *S. agrili* were dissected to assess whether they had been parasitized by *T. planipennisi*. EAB larvae exposed only to *T. planipennisi* and those not parasitized by *S. agrili* were held in the incubator for 10 days to observe for parasitism.

2.2.2. Field trials

In June and July 2009, 10 ♀ *T. planipennisi* and three ♀ *S. agrili* were presented alone and together with 4th instar field-collected EAB larvae inserted into seven healthy young green ash (*Fraxinus pennsylvanica* Marsh.) trees (mean ± SE diameter at 1.5 m = 5.2 ± 0.3 cm) growing in a second-growth, mixed hardwood wetland forest in Central Park, Okemos, Michigan. Three cages were attached to each tree within 3 m of the ground (Fig. 1C). The cages were made of fine fabric screening held ~5–10 cm away from the trunk by metal wire frames attached to each tree. Water in a vial with a cotton wick and streaks of honey on the inside surface of a Petri dish lid were suspended in each cage (Fig. 1D). A section of plastic sheeting was stapled to the tree above each cage to provide shelter during rainstorms (Fig. 1D). Each cage surrounded a ~0.5 m length of trunk in which 10 EAB larvae had been inserted (under flaps of bark, as described above). The larvae were arranged in two "rings" encircling the trunk, each with five larvae, with the rings being ~10 cm apart. The cages were constructed over a 2 day period: EAB larvae were inserted and the wire frames attached on the first day and water, honey, screening and wasps were added on the second day. Duct tape was used to connect the edges of the screen and to secure the screen to the trunk at the top and bottom of the cage. The three treatments were randomly assigned to the three cages on each tree. After two weeks, the caged trunk sections were removed from the field and dissected in the laboratory to determine the fate of each EAB larvae, as described above.

2.3. Short-term parasitism rates

Five ♀ *T. planipennisi* and five ♀ *S. agrili* were exposed to EAB larvae in large sticks (i.e., five larvae per stick) for 27 h to compare short-term parasitism rates (i.e., the percentage of hosts parasitized). The *S. agrili* trials were performed in the parasitized-host acceptability experiment described above. For the *T. planipennisi* trials, five large sticks, each containing five EAB larvae, were placed

Table 1
Summary of experiments. See text for more information.

Research objective *Experiment* Treatment	# Reps	# Hosts per rep	# ♀/♂ *T. planipennisi*	# ♀/♂ *S. agrili*
Dominance assays				
Small sticks (laboratory)				
T. planipennisi	10	5	5/0	NA
S. agrili	5	5	NA	2/0
Both species	5	5	5/0	2/0
Large sticks (laboratory)				
T. planipennisi	12	5	5/0	NA
S. agrili	5	5	NA	2/0
Both species	4	5	5/0	2/0
Living trees (field)				
T. planipennisi	7	10	10/0	NA
S. agrili	7	10	NA	3/0
Both species	7	10	10/0	3/0
Short-term parasitism rates (large sticks, laboratory)				
T. planipennisi for 27 h	5	5	5/0	NA
S. agrili for 27 h	5	5	NA	5/0
Multiparasitism assays (large sticks, laboratory)				
S. agrili-parasitized hosts given to T. planipennisi				
S. agrili added for 27 h then replaced with *T. planipennisi*	5	5	5/0	5/0
S. agrili not added for 27 h before adding *T. planipennisi*	5	5	5/0	NA
T. planipennisi-parasitized hosts given to *S. agrili*				
T. planipennisi added for 5 days then replaced with *S. agrili*	5	5	5/5	2/0
T. planipennisi added for 9 days then replaced with *S. agrili*	5	5	5/5	2/0
T. planipennisi added for 5 days then dissected	5	5	5/5	NA

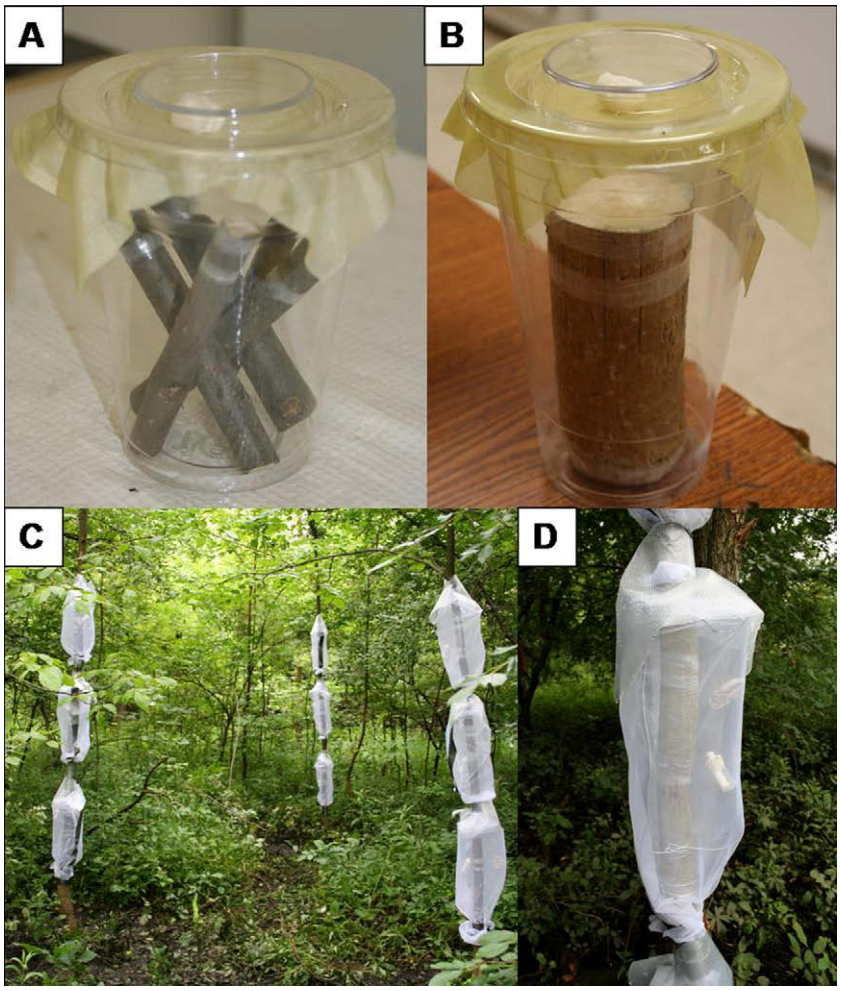

Fig. 1. *T. planipennisi* were presented with larvae inserted into small (A) and large (B) sticks in the laboratory and with larvae inserted into trunks of healthy trees in the field (C and D).

in individual cups as described above. Five ♀ *T. planipennisi* were then added to each cup. The sticks were dissected after 27 h and the larvae were placed in individual Petri dishes lined with moistened filter paper to observe for parasitism, as described above.

2.4. Multiparasitism assays

Two experiments were performed to determine if *T. planipennisi* or *S. agrili* would parasitize 4th instar EAB larvae previously parasitized by the other species (Table 1).

2.4.1. S. agrili-parasitized hosts given to T. planipennisi

Ten large sticks, each containing five EAB larvae, were placed in individual cups as described above. Five ♀ *S. agrili* were immediately added to each of five cups. After 27 h, the *S. agrili* were removed and replaced with five ♀ *T. planipennisi*. The remaining five cups, to which *S. agrili* had not been added, each received five ♀ *T. planipennisi*. All sticks were dissected after 12 days to determine the fate of each EAB larvae, as described above.

2.4.2. T. planipennisi-parasitized hosts given to S. agrili

Fifteen large sticks, each containing five EAB larvae, were placed in individual cups as described above. Five ♀ and five ♂ *T. planipennisi* were immediately added to each of the cups. These were replaced with 2 ♀ *S. agrili* after 5 days in five of the cups and after 9 days in five other cups. These sticks were dissected 7 and 3 days later, respectively (i.e., after 12 days in each case). The fate of each EAB larvae was determined as described above. The remaining five sticks were dissected on day 5 to monitor larval movement as *S. agrili* is known to locate hosts by detecting vibrations under the bark (Wang et al., 2010). These larvae were placed in individual Petri dishes lined with moistened filter paper and observed again on day 9. Data on parasitism by *T. planipennisi* were also collected from these sticks.

2.5. Data collection and analysis

The following data were collected for each replicate (i.e., cup in the laboratory or cage in the field): (1) % of EAB larvae parasitized, (2) % of non-parasitized EAB larvae that were dead, (3) % of non-parasitized EAB larvae that were alive, (4) average number of parasitoid progeny per parasitized larvae for each species. These data were square root-transformed before calculating 95% confidence intervals (Sokal and Rohlf, 1995). Means with non-overlapping confidence intervals are considered significantly different. For comparisons of particular interest, ANOVAs were performed as well ($\alpha = 0.05$). Untransformed means and back-transformed confidence intervals are presented here.

3. Results

3.1. Dominance assays

3.1.1. Laboratory trials

Spathius agrili and *T. planipennisi* exhibited similar rates of parasitism in the laboratory, ~50–60% on average (Table 2), when presented alone with EAB larvae. For both small ($F_{1,13} = 95.81$, $P < 0.0001$) and large ($F_{1,14} = 92.49$, $P < 0.0001$) sticks, *T. planipennisi* parasitized significantly fewer larvae when *S. agrili* was present than when alone; in fact, no larvae were parasitized by *T. planipennisi* when *S. agrili* was present (Table 2). In contrast, *S. agrili* parasitized a similar percentage of larvae in small sticks ($F_{1,8} = 1.04$, $P = 0.34$) and significantly more larvae in large sticks ($F_{1,7} = 8.08$, $P = 0.02$) when *T. planipennisi* was present than when alone (Table 2). While there was no difference in the number of *S. agrili* progeny per host between small and large sticks, significantly more ($F_{1,20} = 7.71$, $P = 0.01$) *T. planipennisi* progeny were produced on average from larvae in large sticks than small sticks (Table 2).

3.1.2. Field trials

Spathius agrili and *T. planipennisi* exhibited similar rates of parasitism in the field (Table 2), when individually presented with EAB

Table 2
Results (mean (95% CI)) from laboratory and field dominance assays in which *T. planipennisi* and *S. agrili* were presented individually and together with 4th instar EAB larvae.

Treatment (i.e., species)	Stick size	*n*	% Parasitized by *S. agrili*	% Parasitized by *T. planipennisi*	% Not parasitized		# *S. agrili* progeny per larva[a]	# *T. planipennisi* progeny per larva[a]
					% Dead	% Alive		
Laboratory trials								
T. planipennisi	Small	10	NA	58.0 (41.1–72.0)	16.0 (0.7–21.6)	26.0 (12.0–35.7)	NA	44.8 (32.2–55.3)
T. planipennisi	Large	12	NA	53.3 (40.3–63.9)	21.7 (2.4–27.9)	25.0 (4.9–33.3)	NA	67.7 (55.2–78.4)
S. agrili	Small	5	60.0 (24.1–95.4)	NA	40.0 (0.7–78.0)	0	6.8 (5.6–8.0)	NA
S. agrili	Large	5	56.0 (47.7–64.3)	NA	28.0 (5.2–50.3)	16.0 (0.3–31.2)	7.0 (4.6–9.5)	NA
Both species	Small	5	76.0 (61.5–90.5)	0	20.0 (3.6–36.1)	4.0 (0–7.0)	8.4 (8.0–8.8)	0
Both species	Large	4	75.0 (64.9–85.2)	0	25.0 (16.2–34.1)	0	6.5 (4.2–8.9)	0
Field trials								
T. planipennisi	NA	7	NA	17.1 (2.0–28.1)	55.7 (40.8–69.6)	27.1 (13.0–39.3)	NA	65.3 (39.2–91.1)
S. agrili	NA	7	17.1 (0–26.7)	NA[b]	52.9 (21.8–78.9)	30.0 (1.3–49.0)	4.5 (3.4–5.7)	NA
Both species	NA	7	12.9 (3.9–20.0)	4.3[c] (0.0–6.8)	80.0 (70.2–89.4)	5.7 (0.3–9.4)	3.9 (1.6–5.9)	55[d]

[a] As the mean numbers of progeny were calculated using the number of parasitized larvae and not the number of replicates, the values for *n* given in the table do not apply.
[b] One larvae was parasitized by *T. planipennisi* (see Section 4).
[c] Two EAB larvae were parasitized by both *S. agrili* and *T. planipennisi*. A third was parasitized by *T. planipennisi* only.
[d] Single record.

Table 3
Results (mean (95% CI)) from multiparasitism assays. For the *Tetrastichus*-parasitized-host trials, *S. agrili* were presented with larvae that had been exposed to *T. planipennisi* for 5 or 9 days. The third treatment consisted of larvae that had been exposed to *T. planipennisi* for 5 days but never to *S. agrili*. For the *Spathius*-parasitized-host trials, *T. planipennisi* were presented with larvae that had or had not been exposed to *S. agrili* for 27 h. See Section 2 for more information.

Treatment	*n*	% Parasitized by *S. agrili* only	% Parasitized by *T. planipennisi* only	% Parasitized by both	% Not parasitized		# *S. agrili* progeny per larva[a]	# *T. planipennisi* progeny per larva[a]
					% Dead	% Alive		
Tetrastichus-parasitized								
S. agrili added after 5 day	5	16.0 (0.3–31.2)	32.0 (4.4–59.0)	4.0 (0–7.0)	40.0 (5.9–73.3)	8.0 (0–15.5)	8.5 (6.8–10.2)	76.2 (57.0–96.6)
S. agrili added after 9 day	5	0	32.0 (5.4–58.0)	8.0 (0–14.0)	28.0 (2.9–52.5)	32.0 (22.0–41.9)	3.5[b]	64.0 (48.1–80.5)
S. agrili not added	5	NA	36.0 (5.3–66.0)	NA	8.0 (0–14.0)	56.0 (34.3–77.5)	NA	56.3 (23.1–92.1)
Spathius-parasitized								
Exposed to *S. agrili*	5	32.0 (22.0–41.9)	8.0 (0.0–15.5)	0	16.0 (3.3–28.4)	44.0 (28.5–59.4)	10.5 (7.9–13.1)	36.5 (31.7–41.5)
Not Exposed to *S. agrili*	5	NA	28.0 (5.2–50.3)	NA	8.0 (0–15.5)	64.0 (41.6–86.2)	NA	86.5 (70.8–102.5)

[a] As the mean numbers of progeny were calculated using the number of parasitized larvae and not the number of replicates, the values for *n* given in the table do not apply.
[b] Single record.

larvae. *T. planipennisi* parasitized fewer larvae when *S. agrili* was present than when alone (Table 2), but this difference was not significant ($F_{1,12} = 2.85$, $P = 0.12$). Similarly, there was no difference in the percentage of larvae parasitized by *S. agrili* when *T. planipennisi* was present compared to when alone ($F_{1,12} = 0.20$, $P = 0.66$). Two larvae were parasitized by both species. One larva exposed to *S. agrili* alone was unexpectedly parasitized by *T. planipennisi*, most likely by an established female (i.e., parasitoid releases had been made nearby) during the 24 h period between larval insertion and cage completion. Due to this potential source of contamination, results from the field trials should be interpreted with caution.

3.2. Short-term parasitism rates

On average, *S. agrili* parasitized 32% of larvae within 27 h (Table 3) whereas *T. planipennisi* parasitized none within that amount of time (data not shown), a statistically significant difference ($F_{1,8} = 151.42$, $P < 0.0001$).

3.3. Multiparasitism assays

3.3.1. S. agrili-parasitized hosts given to T. planipennisi

No larvae previously parasitized by *S. agrili* showed signs of parasitism by *T. planipennisi* (Table 3).

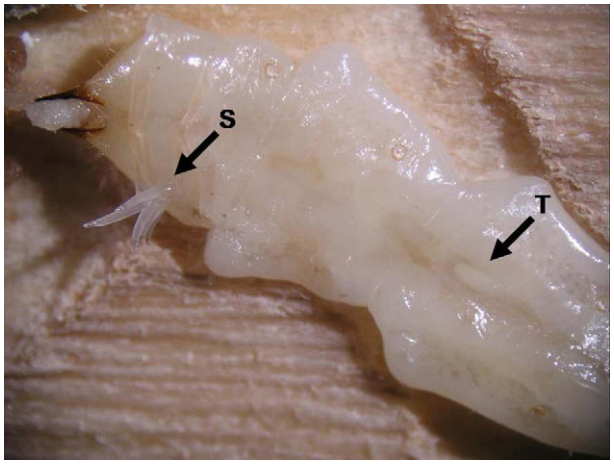

Fig. 2. The posterior end of an EAB larva parasitized by both *S. agrili* (external cluster of eggs, S) and *T. planipennisi* (internal larva, T).

3.3.2. T. planipennisi-parasitized hosts given to S. agrili

The parasitism rate of *S. agrili* was lower for larvae previously exposed to *T. planipennisi* (Table 3) than for non-parasitized larvae (Table 2) and most of the larvae accepted by *S. agrili* were those not previously parasitized by *T. planipennisi* (Table 3). However, *S. agrili* did parasitize three previously-parasitized larvae (i.e., one and two after the 5 and 9 days exposures to *T. planipennisi*, respectively) (Table 3, Fig. 2). Because the first dually parasitized larva was destroyed during dissection to determine if it had been parasitized by *T. planipennisi*, the fate of the parasitoid progeny could not be determined. For the remaining two dually parasitized larvae, the *S. agrili* progeny, which were eggs or 1st instar larvae at the time of collection, failed to complete development before the *T. planipennisi* progeny exited the hosts. Stick dissections revealed that all larvae were active after 5 days of exposure to *T. planipennisi* and several were active after 9 days, including two within which parasitoids were visible.

4. Discussion

4.1. Dominance assays

While *S. agrili* and *T. planipennisi* exhibited similar parasitism rates when presented alone with EAB larvae for 12 days or more, *S. agrili* was more efficient at locating and parasitizing hosts within the first 27 h. This may explain why *S. agrili* out-competed *T. planipennisi* when the two species were presented with EAB larvae together. This was particularly evident in the laboratory where there was no evidence of parasitism by *T. planipennisi* when *S. agrili* was present. In the field, however, *T. planipennisi* parasitized larvae in the presence of *S. agrili* on three occasions (although two of those larvae were also parasitized by *S. agrili*), possibly because the caged enclosures in the field were larger than the cup enclosures in the laboratory and because the ratio of *T. planipennisi* to *S. agrili* was higher in the field (3.33) than in the laboratory (2.5). It is interesting to note that the parasitism rate exhibited by *S. agrili* increased when *T. planipennisi* was present, at least in the laboratory when large sticks were used. However, further research is needed to confirm this observation.

4.2. Multiparasitism assays

Spathius agrili will parasitize larvae previously parasitized by *T. planipennisi* but apparently not the reverse. This is not surprising

considering that larvae parasitized by *T. planipennisi*, a koinobiont, remain active for about a week (J.J.D., unpublished data) while those parasitized by *S. agrili*, an idiobiont, are paralyzed by the female before ovipositioning. It is likely that both species locate their hosts by detecting feeding vibrations. If so, larvae parasitized (i.e., paralyzed) by *S. agrili* would be undetectable to *T. planipennisi*. While none of the *S. agrili* progeny on larvae previously parasitized by *T. planipennisi* completed development, *T. planipennisi* progeny were apparently unaffected by the presence of *S. agrili*. However, it remains unclear which species would "win" if the two species were to parasitize the same larva simultaneously. Because *S. agrili* eggs deposited on larvae previously parasitized by *T. planipennisi* are essentially wasted, it is in the interest of *S. agrili* to avoid such larvae. Our results suggest that while *S. agrili* will occasionally accept larvae previously parasitized by *T. planipennisi*, the species prefers healthy larvae (see Tables 2 and 3).

4.3. Establishment implications

Efforts are underway in Michigan and surrounding states to establish populations of *S. agrili* and *T. planipennisi* in the field. To facilitate successful establishment, we recommend releasing the two species separately in space or time to limit the antagonistic interactions observed in this study. This seems particularly prudent given that parasitoid establishment rates are generally higher in single-species introductions than in multiple-species introductions (Ehler and Hall, 1982; Denoth et al., 2002). However, the implications of our results are somewhat limited by the fact that nothing is known about parasitoid ratios under natural conditions. Furthermore, it is difficult to predict how the two species will interact in nature based on a study in which the parasitoids were confined to small enclosures with limited resources. Long-term monitoring efforts will be needed to determine if *S. agrili* and *T. planipennisi* populations can coexist in North America forests. However, it is encouraging to note the distributions of these species overlap at some sites in China (Liu et al., 2003; L.S.B., unpublished data).

4.4. Methodology considerations

One finding from this study is of particular importance to rearing *T. planipennisi* in the laboratory. We found that about 20 more offspring on average were produced per host when large sticks were used instead of small sticks. We suspect that large sticks retain moisture better than small sticks, and that moisture facilitates ovipositioning. Although the moisture content of intact trees is even higher, the average number of offspring per larva from intact trees and large sticks was similar. However, it is interesting to note the highest number of offspring recorded from a single larva (i.e., 172) occurred in an intact tree in the field. The method described in this paper for inserting larvae into trees may have utility in future studies. However, many more larvae died when inserted into living trees in the field than when inserted into sticks in the laboratory. We attribute these deaths to drowning as the insertion points were often waterlogged. Any modifications to the method that reduce water accumulation (e.g., girdling) will likely enhance larval survivorship. Another important change to the method would be to add the cages immediately after inserting the larvae. We unexpectedly recovered a larvae parasitized by *T. planipennisi* from a cage to which only *S. agrili* had been added. This most likely represents parasitism by a female released nearby, particularly considering that a female was observed on that very tree the same day the larvae were inserted.

4.5. Conclusions

Even when outnumbered by a factor of 2.5 in the laboratory and 3.3 in the field, *S. agrili* excluded or nearly excluded *T. planipennisi*. This can be largely attributed to the fact that *S. agrili* is much more efficient at locating hosts. Many questions need to be answered before we can fully understand the implications of these results. For example, the relative abilities of the two species to tolerate seasonal fluctuations in climate will determine their ranges and potentials for interaction. Furthermore, the extent to which the two species partition resources in space and time will determine the degree to which they encounter one another. Research is underway to answer these and other important questions. In the meantime, efforts should be made to minimize interactions between the species at release sites.

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References

Bauer, L.S., Liu, H., Miller, D.L., Gould, J., 2008. Developing a classical biological control program for *Agrilus planipennis* (Coleoptera: Buprestidae), an invasive ash pest in North America. Newsletter of the Michigan Entomological Society 53, 38–39.

Denoth, M., Frid, L., Myers, J.H., 2002. Multiple agents in biological control: improving the odds? Biological Control 24, 20–30.

Ehler, L.E., Hall, R.W., 1982. Evidence for competitive exclusion of introduced natural enemies in biological control. Environmental Entomology 11, 1–4.

Haack, R.A., Jendek, E., Liu, H., Marchant, K.R., Petrice, T.R., Poland, T.M., Ye, H., 2002. The emerald ash borer: a new exotic pest in North America. Newsletter of the Michigan Entomological Society 47, 1–5.

Liu, H., Bauer, L.S., Gao, R., Zhao, T., Petrice, T.R., Haack, R.A., 2003. Exploratory survey for the emerald ash borer, *Agrilus planipennis* (Coleoptera: Buprestidae), and its natural enemies in China. The Great Lakes Entomologist 36, 191–204.

Liu, H., Bauer, L.S., Miller, D.L., Zhao, T., Gao, R., Song, L., Luan, Q., Jin, R., Gao, C., 2007. Seasonal abundance of *Agrilus planipennis* (Coleoptera: Buprestidae) and its natural enemies *Oobius agrili* (Hymenoptera: Encyrtidae) and *Tetrastichus planipennisi* (Hymenoptera: Eulophidae) in China. Biological Control 42, 61–71.

Mills, N., 2006. Interspecific competition among natural enemies and single versus multiple introductions in biological control. In: Brodeur, J., Boivin, G. (Eds.), Trophic and Guild Interactions in Biological Control. Springer, Dordrecht, pp. 191–220.

Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in Biological Research. W. H. Freeman and Company, New York. 887pp.

Wang, X.-Y., Yang, Z.-Q., Gould, J.R., Wu, H., Ma, J.-H., 2010. Host-seeking behavior and parasitism by *Spathius agrili* Yang (Hymenoptera: Braconidae), a parasitoid of the emerald ash borer. Biological Control 52, 24–29.

Yang, Z.-Q., Strazanac, J.S., Marsh, P.M., Van Achterberg, C., Choi, W.-Y., 2005. First recorded parasitoid from China of *Agrilus planipennis*: a new species of *Spathius* (Hymenoptera: Braconidae: Doryctinae). Annals of the Entomological Society of America 98, 636–642.

Yang, Z.-Q., Srazanac, J.S., Yao, Y.-X., Wang, X.-Y., 2006. A new species of emerald ash borer parasitoid from China belonging to the genus *Tetrastichus* Haliday (Hymenoptera: Eulophidae). Proceedings of the Entomological Society of Washington 108, 550–558.