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# Mortality factors affecting *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae) eggs in the native and invaded ranges

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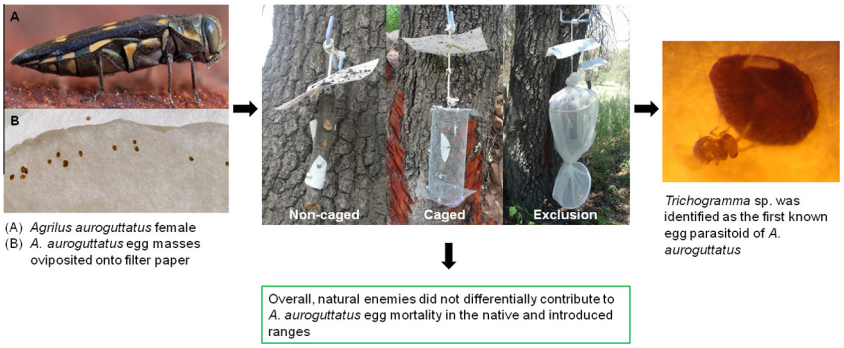
## HIGHLIGHTS

- *Agrilus auroguttatus* is an invasive beetle killing native California (CA) oaks.
- Native to Arizona (AZ), its success in CA may be due to a lack of natural enemies.
- Mortality of *A. auroguttatus* sentinel eggs deployed in AZ and CA were compared.
- Natural enemies did not differentially contribute to egg mortality in AZ and CA.
- The first known egg parasitoid of *A. auroguttatus* was collected during this study.

## GRAPHICAL ABSTRACT

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## ABSTRACT

An absence of diverse and coevolved natural enemies may explain the high levels of oak mortality caused by an invasive wood boring beetle, *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae), in California (CA). A field study was conducted to test the enemy release hypothesis for a single guild of natural enemies by comparing mortality factors affecting *A. auroguttatus* sentinel eggs deployed in both native (southern Arizona [AZ]) and introduced ranges (southern CA). The percentage of eggs attacked by natural enemies did not differ between sites, which does not support the enemy release hypothesis for this life stage. Although the predominant cause of mortality to sentinel eggs deployed in CA and AZ was due to factors other than natural enemy activity, chewed, missing, and parasitized eggs contributed to as much as 16% and 24% of sentinel egg mortality in CA and AZ, respectively. In addition, the first known egg parasitoid of *A. auroguttatus* was collected during this study from a single egg deployed in AZ, and was identified as *Trichogramma* sp. using molecular techniques. This parasitoid is a generalist, and therefore not suitable for use in a classical biological control program against *A. auroguttatus* in CA. A continuation of this study is needed across a larger number of field sites and over a longer period of time to optimize the potential detection of host specific egg parasitoids for potential introduction into CA as part of a future classical biological control program, and to better quantify natural enemy impacts on *A. auroguttatus* eggs.



## 1. Introduction

The goldspotted oak borer (GSOB), *Agrilus auroguttatus* Schaeffer (Coleoptera: Buprestidae), is an invasive pest that was accidently introduced into southern California's (CA) oak forests. *A.*

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*auroguttatus* is native to southern Arizona (AZ) and was likely introduced into CA through the transportation of infested oak firewood. This beetle was initially detected in San Diego County, CA in 2004 (Westcott, 2005), but was not associated with the area-wide decline and mortality of indigenous oaks until 2008 (Coleman and Seybold, 2008a). Aerial surveys of oak mortality since 2002 suggest that the incipient population of *A. auroguttatus* was introduced into southern CA several years prior to its initial detection in 2004, and is expanding its range (Coleman et al., 2012a).

Unlike most *Agrilus* species which are associated with oak trees already in decline (e.g., *Agrilus bilineatus* [Weber] in the northeastern United States and *Agrilus bigutattus* [Fabricius] in Europe), *A. auroguttatus* is the primary cause of mortality to coast live oak (*Quercus agrifolia* Nèe), California black oak ( *Quercus kelloggii* Newb.), and canyon live oak (*Quercus chrysolepis* Liebm.) in southern CA (Coleman et al., 2012a). Mortality of these native species is estimated at >22,000 trees within the 212, 460 ha infestation zone, and is caused by extensive larval feeding damage to the phloem/xylem interface (Coleman et al., 2012a). *Agrilus auroguttatus* preferentially attacks the main stem of large diameter (>12 cm at breast height) trees in the red oak group (section *Lobatae*), although minor injury to the white oak, *Quercus engelmanni* Greene (section *Quercus*), has been observed (Coleman and Seybold, 2011). Symptoms of infestation include crown-thinning and dieback, D-shaped exit holes, bark staining, and woodpecker damage (Hishinuma et al., 2011).

The oak forests of southern CA are largely composed of species from the red oak group, which hold dominant and co-dominant positions in the canopy, and are highly vulnerable to *A. auroguttatus* infestation (Coleman and Seybold, 2008b). The widespread loss of these foundation species is anticipated to result in detrimental effects to southern California's oak savanna and mixed conifer ecosystems by affecting energy and nutrient inputs, hydrology, food webs, and biodiversity (Ellison et al., 2005). Biodiversity in these unique forests is expected to be negatively impacted due to the loss of habitat and food resources for native wildlife (McShea et al., 2007). Additionally, the accumulation of dead oak litter from trees killed by *A. auroguttatus* will alter the fuel load in affected areas, increasing the probability and severity of wildfires (Coleman and Seybold, 2008b).

Strategies under investigation for *A. auroguttatus* management include topical and systemic insecticide use, wood solarization (i.e., wrapping infested wood in plastic and heating in the sun to kill larvae or pupae in wood, or to trap and kill adults as they emerge), and grinding infested wood (Coleman and Seybold, 2008a). These strategies are designed for either treating individual, privately owned trees (e.g., insecticides), or for slowing expansion of the infestation zone via the movement of infested firewood (e.g., wood solarizing and grinding). Currently, there is no functional strategy to manage this beetle in a forest environment. While the efficacy of insecticide use for *A. auroguttatus* management is currently being evaluated, pesticide use to protect trees in forest stands is neither cost effective, sustainable, or environmentally appropriate. The development of a classical biological control program for suppressing *A. auroguttatus* populations with co-evolved host specific parasitoids is an appealing forest management strategy in southern CA since it has the potential to be permanent, widespread, cost-effective, and environmentally safe.

Classical biological control has been an effective tool for lowering populations of non-native forest pests (Hajek, 1999; Ryan et al., 1978; Roland and Embree, 1995; Van Driesche et al., 2010). The recent mortality of millions of native ash trees (*Fraxinus* spp.) by the invasive emerald ash borer (*Agrilus planipennis* Fairmaire) in North America has highlighted the destructive capabilities of introduced woodborers on native forest ecosystems. Management of *A. planipennis* in the northeast has focused on biological control since containment through early detection, quarantine, and infested tree removal has had little success (Cappaert et al., 2005; Duan et al., 2011). Egg parasitoids have shown potential as biological control agents of *A. planipennis* (Liu et al., 2007). These natural enemies, should they exist for *A. auroguttatus*, are of high interest for use in the emerging biological control program for this pest in CA due to the efficiency of producing eggs in the laboratory as compared to other life stages (e.g., larvae), which in the absence of artificial diet, require cut logs for rearing, which are not effective for rearing larvae through to adults.

In its native range in southern AZ, *A. auroguttatus* is not pestiferous and exhibits behavior similar to other *Agrilus* species that preferentially attack trees already in decline (Coleman et al., 2012a). The rarity of *A. auroguttatus* specimen collections from museum and field surveys, and lack of data in the economic entomology literature denotes the relative insignificance of this beetle regarding oak forest health in southern AZ (Coleman and Seybold, 2011). In comparison, the elevated levels of oak decline and mortality in southern CA could be due to the new association of *A. auroguttatus* with ecologically naïve hosts and a lack of host-specific natural enemies in the introduced range (Coleman and Seybold, 2011).

The enemy release hypothesis predicts that alien species introduced into a new region should experience reduced impacts from natural enemies which will lead to an increase in their distribution and abundance (Roy et al., 2011). This hypothesis is the theoretical foundation of classical biological control (Liu and Stiling, 2006), and has been commonly used to explain the success of invasive pests, especially plant and arthropod species, when they are uncoupled from their co-evolved natural enemies (Cincotta et al., 2009; Georgiev et al., 2007; Keane and Crawley, 2002; Koyama and Majerus, 2008). The disproportionate population densities of *A. auroguttatus* in the introduced range suggest that the success of this woodborer in southern CA could be due, in part, to release from co-evolved natural enemies.

Here, we test the enemy release hypothesis on a single guild of natural enemies by comparing the mortality factors of *A. auroguttatus* sentinel eggs deployed in both native (southern AZ) and introduced ranges (southern CA). This study will help to determine the potential role natural enemies play on the population dynamics of this beetle in CA and AZ. Results from these field surveys with deployed *A. auroguttatus* eggs provide useful data for identifying surveying techniques and potential egg parasitoids for future use in a classical biological control program against *A. auroguttatus* in southern CA.

## 2. Materials and methods

### 2.1. Study sites

Field studies were conducted in the native (Arizona [AZ], USA) and introduced range (southern California [CA], USA) of *A. auroguttatus*. Two oak forest field sites, one in each state, were selected from counties where this beetle had been previously collected (Coleman and Seybold, 2011). Site 1 (31°43′N/110°43′W; 1509–1523 m), part of the native range of *A. auroguttatus*, was an approximately 1 hectare plot located at Gardner Canyon in the Santa Rita Mountains, Pima County, AZ, USA. Site 2 (33°02′N/116°35′W; 1277–1296 m), part of the invaded range in southern CA, an approximately 1.4 ha plot, was located at William Heise County Park, San Diego County, CA, USA. At each site, six trees (infested with larval *A. auroguttatus*) were selected for deployment of *A. auroguttatus* sentinel eggs to measure mortality factors on this life stage in the native and invaded ranges. Trees were considered infested if symptoms described in Hishinuma et al. (2011) such

as larval galleries and exit holes were present. At each study tree, sentinel eggs were deployed in non-caged, caged, and exclusion treatments to determine whether egg mortality rates were affected by increasingly limiting natural enemy access to sentinel eggs. The deployment of *A. auroguttatus* eggs at each site was conducted over an 8 week period during July–September 2012.

### 2.2. Preparation of A. auroguttatus sentinel egg masses

*Agrilus auroguttatus* sentinel eggs were produced in the laboratory by allowing field-collected adults to deposit their eggs onto coffee filter paper. *Agrilus auroguttatus* adults were reared from infested *Q. agrifolia and Q. kelloggii* trees that were felled in April 2012 at William Heise County Park, Julian, CA, cut into rounds (approximately 30 × 60 cm), and placed inside 15 emergence tents (also located at William Heise County Park). During June to August 2012, adults were collected daily from emergence tents (1.83 × 1.83 × 1.83 m Lumite® screen portable field cages, Bioquip Products, Rancho Dominguez, CA) containing approximately 15 *A. auroguttatus*-infested oak rounds. From these daily collections, a mixture of 10–15 reproductively mature males and females were introduced into 2.13 L hand-grip rearing containers (11.7 × 12.1 × 18.1 cm, Candy Concepts Inc., Pewaukee, WI) with a 6 cm diameter ventilation hole that was covered with fine metal mesh screen. Adults that died were replaced with other reproductively mature adults to maintain a consistent number of 10–15 adults in each rearing container. A total of 10 rearing containers were held under ambient laboratory conditions (14:10 L:D, 24 ± 4 °C, 35 ± 5% RH) and contained host plant leaves (*Q. kelloggii*), moist cotton wick as a water source, and standard white coffee filter paper (11.1 cm diameter base, Ambiance™, Amerifoods Trading Co., Los Angeles, CA) as an oviposition substrate. The coffee filter paper was cut into approximately 10 cm diameter rounds and placed directly underneath the rearing container lid. A metal mesh screen was placed on top of the filter paper which held the paper in place, secured the ventilation hole, and provided adults with a textured substrate for gripping while ovipositing. This method of acquiring eggs has been used successfully for research on emerald ash borer (*A. planipennis*) biological control (Yang et al., 2012). Coffee filters were placed inside each rearing container for 1–2 days, removed, and quarter rounds with eggs were excised for placement onto cut oak branches (see below for more details on egg deployment). Sectioned coffee filters with eggs were numbered, eggs were counted before field deployment, and were 2–3 days old at the time of field placement.

### 2.3. Field deployment of sentinel A. auroguttatus egg masses

*Agrilus auroguttatus* eggs on sectioned and labeled coffee filters were attached to cut oak branches. Cut oak branches were collected in the field by cutting branches (approximately 6 cm in diameter × 18 cm in length) from native AZ (*Quercus emoryi*) and CA oaks (*Q. agrifolia*) using a pruning saw. Egg-infested oak branches were made by attaching two quarter round egg papers (these were numbered and the number of eggs deployed per treatment were recorded) to an oak branch with nickel plated thumbtacks. An eye-loop bolt was screwed into the top of each cut oak branch for hanging (approximately 12 cm from trunk) on hooks that were attached to the trunks of selected trees.

Oak branches with *A. auroguttatus* eggs were randomly assigned to one of three treatments: (1) non-caged branches, where *A. auroguttatus* eggs were fully exposed to natural enemies (parasitoids and predators), (2) caged branches, where branches were suspended inside metal mesh cylinders to exclude large generalist predators, but preferentially allow parasitoid access, and (3) exclusion cages that used the same cage from (2) above, which were fully enclosed within a fine mesh bag to exclude all natural enemies. All three treatment types were individually suspended on each of six *A. auroguttatus*-infested trees, for a total of 18 egg-infested oak branches deployed at each field site.

Wire mesh cages used in the caged treatment were made by forming a tube (approximately 24 cm length and 15 cm diameter) out of 0.3 cm hardware cloth and securing with zip-ties. At the top and bottom of each tube, a flat square of hardware cloth (approximately 12 × 12 cm) was attached using zip-ties, thereby enclosing the tube with an *A. auroguttatus* egg-infested oak branch inside. Egg-infested oak branches were held inside each cage using an eye-loop bolt that was screwed in from the outside of the cage, through an opening in the mesh of the top square of hardware cloth, then into the egg-infested oak branch. The bottom square of hardware cloth was attached after an egg-infested oak branch was in place. Egg-infested oak branches were positioned in the center of the cage so that no contact was made between the branch and cage walls.

The exclusion treatment was made by placing a caged treatment into an "exclusion bag". Exclusion bags (approximately 45 cm in length × 30 cm in diameter) were made by sewing white no-see-um netting (lightweight no-see-um fine polyester netting, approximately 100 holes per sq. cm, Skeeta, Bradenton, FL). Exclusion bags were closed with a drawstring thereby fully enclosing the hardware cloth cage. Each treatment type was hung from the eye-loop bolt onto a selected tree using a screw-in ladder hook (19 cm length × 4.5 cm height, Crawford® Ladder Hook (Ss11–50), Lehigh Consumer Products, LLC, Rye, NY) and polypropylene rope. Ladder hooks were screwed approximately 1.5 m above the ground into the trunk of a selected *A. auroguttatus*-infested tree. For each treatment type, a flat white sticky trap (19.5 × 16 cm) was hung through the rope connecting the eye-loop bolt to the ladder hook and was situated above the suspended oak branch bearing the *A. auroguttatus* egg papers. Sticky traps were used to discourage potential egg predation by ants.

### 2.4. Retrieval and rearing of A. auroguttatus sentinel egg masses

Sentinel eggs were deployed for 7 days and replaced on this weekly schedule at both study sites for 8 consecutive weeks. Eggs on each egg paper were counted immediately after collection to determine the number of missing eggs per replicate for each treatment. Egg papers collected from field sites were placed immediately into sterile 100 mm × 15 mm polystyrene Petri dishes, sealed with Parafilm M® (Peniney Plastic, Chicago, IL), and transported under valid permit to the Insectary and Quarantine facility at the University of California, Riverside. Egg papers were stored under ambient laboratory conditions (14:10 L:D, 23 ± 2 °C, 30% RH) and checked every other day for three weeks for parasitoid emergence. Three weeks was considered a sufficient time period for egg incubation following field collections because under laboratory conditions (14:10 L:D, 23 ± 2 °C, 30% RH), *A. auroguttatus* neonate larvae emerge 10–15 days after oviposition (VML, unpublished data).

Three weeks post-collection, eggs on each piece of coffee filter paper were examined under a dissecting microscope and assigned to one of 6 categories: (1) hatched – a visible *A. auroguttatus* emergence hole, (2) unhatched – no visible emergence hole, (3) non-viable – no melanization and shriveled, (4) chewed - fragments of egg chorion remaining on paper, (5) parasitized – visible parasitoid with associated exit hole or parasitoid life stage inside the egg, or (6) missing – no egg or chorion remaining on papers. All unhatched eggs were dissected under a stereomicroscope to determine whether under-developed *A. auroguttatus* larvae or parasitoids were present. If unhatched or hatched insects could not be identi-

fied by morphology, DNA was extracted from individuals and analyzed.

### 2.5. DNA extraction and analysis of parasitoids collected from A. auroguttatus eggs

DNA was extracted from unidentified individuals collected from *A. auroguttatus* eggs using the EDNA HiSpEx tissue kit (Saturn Biotech, Perth, Australia), following the manufacturer's protocol for 1 $mm^3$ of tissue, but reducing the volume of each kit component to one quarter of that suggested, thereby resulting in a final 25 μL extraction. DNA isolation using this kit involves simple mixing of three proprietary solutions, no grinding of the specimen, and incubation at 95 °C for 30 min. Two adult parasitoids that emerged from *A. auroguttatus* eggs collected in AZ were tentatively identified as Trichogrammatidae by morphology. Therefore, PCR was performed on these and 5 unidentified pre-pupae found inside blackened, unhatched *A. auroguttatus* eggs also collected in AZ using the "ITS2-forward" and "ITS2rev-Trich" primers according to the protocol developed by Stouthamer et al. (1999). Representative sequences were deposited in GenBank (Benson et al., 2008).

### 2.6. Statistical analyses

Percentage egg mortality for each category was calculated based on the total number of eggs deployed each week onto individual egg-infested branches (Duan et al., 2011). Effects of site (two levels) and treatment (three levels) were analyzed using nested ANOVA. Because the species identity of the six trees selected at the CA site differed from those at the AZ site, a nested ANOVA was used with the level of tree nested under the level of site. The percentage of unhatched, non-viable, and missing eggs did not meet normality assumptions, and were transformed and analyzed on a natural logarithm scale. The percentage of chewed eggs was analyzed using the Cochran–Mantel–Haenszel test since data transformation was insufficient to meet normality assumptions. Finally, Fisher's exact test was used to determine whether the combinations of site and treatments were independent of percentage parasitization. All statistical analyses were conducted at the 0.05 level of significance and were performed using SAS 9.2 (SAS Institute Inc., 2008).

## 3. Results

The fates of *A. auroguttatus* sentinel eggs deployed in Gardner Canyon, Pima County, AZ, and William Heise County Park, San Diego County, CA are shown in Tables 1 and 2, respectively. The majority of eggs deployed in AZ and CA hatched (56–72%), while 9–18% were non-viable, and 9–13% simply did not hatch. Unlike healthy eggs which are rounded, smooth, and begin to melanize several hours following oviposition, non-viable eggs were easily distinguished from healthy eggs by their shriveled, sunken appearance, and lack of melanization. Non-viability was observed before and after AZ and CA egg deployment, and was most likely due to infertility or possibly egg damage during oviposition. During stereoscope inspection, fully formed first instar larvae were observed inside unhatched eggs that were deployed in the AZ and CA sites. Unsuccessful hatching of eggs may be attributed to environmental conditions such as unfavorable temperature and/or humidity either in the field or laboratory. The percentage of missing (3–23%) and parasitized (0.2%) eggs was greater in AZ than in CA (2–14% and 0%, respectively), while the percentage of chewed eggs was marginally greater in CA (0.5–2%) than in AZ (0.1–1%). Missing eggs were considered to be the result of predation by natural enemies (e.g., predators that could fly and land directly onto egg papers) since *A. auroguttatus* eggs that are oviposited onto coffee filter paper do not easily fall off, even during handling in windy and rainy conditions.

**Table 1**
Fates of *Agrilus auroguttatus* sentinel eggs deployed at Gardner Canyon, Pima County Arizona.

| Fate of deployed eggs | Non-caged | Caged | Exclusion |
|---|---|---|---|
| Total No. of eggs deployed | 3237 | 2554 | 1637 |
| No. hatched eggs (%) | 1850 (57.2) | 1683 (65.9) | 1132 (69.2) |
| No. parasitized eggs (%) | 7 (0.2) | 0 (0) | 0 (0) |
| No. unhatched eggs (%) | 304 (9.4) | 295 (11.6) | 162 (9.9) |
| No. non-viable eggs (%) | 301 (9.3) | 408 (16.0) | 294 (18.0) |
| No. missing eggs (%) | 745 (23.0) | 148 (5.8) | 48 (2.9) |
| No. chewed eggs (%) | 30 (1.0) | 20 (0.8) | 1 (0.1) |

**Table 2**
Fate of *Agrilus auroguttatus* sentinel eggs deployed at William Heise County Park, San Diego California.

| Fate of deployed eggs | Non-caged | Caged | Exclusion |
|---|---|---|---|
| Total No. of eggs deployed | 2492 | 1991 | 1193 |
| No. hatched eggs (%) | 1713 (68.7) | 1106 (55.5) | 863 (72.3) |
| No. parasitized eggs (%) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| No. unhatched eggs (%) | 329 (13.2) | 246 (12.4) | 119 (10.0) |
| No. non-viable eggs (%) | 378 (15.2) | 328 (16.5) | 187 (15.7) |
| No. missing eggs (%) | 60 (2.4) | 281 (14.1) | 24 (2.0) |
| No. chewed eggs (%) | 12 (0.5) | 31 (1.6) | 0 (0.0) |

### 3.1. Parasitoid identification from genetic analyses

Parasitism was only observed from a single egg paper that was deployed into a non-caged treatment in AZ. Following DNA extraction and amplification, sequences of the ITS2 gene region identified all seven parasitoids as identical (GenBank accession KC512817). A BLAST search (Zhang et al., 2000) identified the parasitoids as a *Trichogramma* sp. with greater than 99% certainty. Comparison to a privately held database of *Trichogramma* ITS2 sequences (Richard Stouthamer, University of California, Riverside, unpublished data) produced a 100% match with a currently undescribed *Trichogramma* sp. that had previously been collected from unidentified Lepidoptera host eggs found in date palms (*Phoenix* sp.) and *Eriogonum* sp. in the Coachella Valley, Riverside County, CA.

### 3.2. Comparison of egg deployment treatments

There was no significant difference ($P > 0.05$) between treatment type or site for the percentage of hatched and unhatched eggs deployed in AZ and CA. The percentage of non-viable eggs was significantly different between non-cage and exclusion treatments deployed in both CA and AZ ($F_{2,20} = 3.69$, $P = 0.04$), with exclusion treatments having a higher percentage of non-viable eggs. Additionally, the percentage of non-viable eggs was significantly greater in the CA site than in the AZ site ($F_{1,10} = 6.11$, $P = 0.03$). The percentage of missing eggs between treatments was significantly greater in non-cage treatments than in exclusion treatments ($F_{2,20} = 5.33$, $P = 0.01$), while site effects were only marginally significant ($F_{1,10} = 4.79$, $P = 0.05$). Pair-wise treatment contrasts on the percentage of chewed eggs showed a significant difference between cage and exclusion treatments ($\chi^2 = 12.31$, df = 1, $P = 0.0005$), with cage treatments having the highest percentage of chewed eggs. The percentage of chewed eggs was also significantly greater in non-cage treatments than in exclusion treatments ($\chi^2 = 9.79$, df = 1, $P = 0.002$). However, there was no significant difference in the percentage of chewed eggs between sites. Fisher's exact test showed a strong association in the percentage of

parasitization for the combination of site and treatments ($P < 0.0001$). This can be attributed to the single collection of parasitized eggs from one egg card deployed in a non-caged treatment in AZ. Finally, there was no significant difference ($P > 0.05$) between treatments or site for the percentage of eggs damaged by natural enemies (No. of chewed eggs + No. of missing eggs + No. of parasitoids found/total No. of eggs deployed) deployed during this study.

## 4. Discussion

Understanding the mechanisms behind the contrasting effects of *A. auroguttatus* on oak survivorship in its native and introduced range has been a primary goal in several *A. auroguttatus* research programs (Coleman and Seybold, 2008b, 2011; Coleman et al., 2011, 2012a,b). Hypotheses explaining high levels of tree injury and mortality by *A. auroguttatus* in CA include an absence of diverse and co-evolved natural enemies, and variation in host resistance within the native and introduced range (Coleman and Seybold, 2011). The invasiveness of *A. auroguttatus* in CA is likely attributed to a combination of natural enemy release, low host resistance, and potentially other unknown factors (i.e., high host suitability). However, given the limited number of studies, it is difficult to determine the extent of each of these individual factors, alone or in combination, and their effect on the invasion success of *A. auroguttatus* in CA.

Although initial natural enemy surveys focusing on larval and pupal parasitoids had detected greater species richness and abundance in AZ than in CA (Coleman et al., 2012b), this was not observed in this study for eggs, as the combination of overall factors affecting egg mortality from natural enemies (i.e., missing, chewed, and parasitized eggs) did not differ between locations. This study was conducted over a relatively short, eight week survey period from a single site in both AZ and CA which could have contributed to our low detection of egg parasitoids, just one *Trichogramma* sp., which was recovered from just seven *A. auroguttatus* eggs in AZ.

These results indicate that although predation (i.e., chewed and missing eggs) and parasitism of GSOB eggs was minor compared to other mortality factors (i.e., non-viable and unhatched eggs), natural enemy activity may be an important influence on egg-stage population densities. In AZ, as much as 24% of sentinel egg mortality (from chewed, missing, and parasitized eggs) was contributed to natural enemies. In CA, egg mortality from natural enemies was lower, accounting for 16% of all mortality factors. While no egg parasitoids of *A. auroguttatus* were detected in CA during this study, an unidentified psocopteran was observed inside a chewed *A. auroguttatus* egg. Although most psocids are herbivores or detritivores, a few are partial predators that consume insect eggs and possibly scale insects (Baz, 2008). An in depth study examining egg-stage mortality factors over several generations and sites would help to determine the extent of these predator impacts on *A. auroguttatus* populations in both the native and introduced range.

Importantly, the first known egg parasitoid of *A. auroguttatus* was collected during this study. Prior to this work, egg parasitoids of *A. auroguttatus* were unknown. The parasitoid collected was identified as *Trichogramma* sp., and was obtained from sentinel eggs deployed in the native range. Investigation into the identity of this species (using ITS2 sequences) found a previous collection record of this parasitoid from Lepidoptera eggs collected in Riverside County, California (Richard Stouthamer, University of California, Riverside, pers. comm.). The low percentage of parasitism by this parasitoid, and its previous collection records from non-Coleoptera hosts indicates that this species is likely a generalist that opportunistically parasitized sentinel GSOB eggs in AZ.

The detection of very few egg parasitoids in this study could be the result of inadequate surveying techniques, an insufficient search range or duration, or simply a lack of this particular guild of natural enemies in the native and introduced range. Egg parasitoids of *Agrilus* spp. can be very challenging to locate due to their small size and the concealed locations of their host's eggs, which are often laid under loose bark or in crevices of bark (Duan et al., 2012). *Agrilus auroguttatus* eggs are small (approximately 1 mm in width), turn a brownish color 2–3 days after oviposition, and are laid deep inside the cracks and crevices of oak bark (Lopez and Hoddle, 2013). Additionally, the preferred oak hosts of *A. auroguttatus* have dark, rough, hard bark that does not easily flake away, which makes locating *A. auroguttatus* eggs in the field very difficult. Since 2008, *A. auroguttatus* eggs have only been detected in the field on a single occasion in which a hatchet was required to remove bark pieces for inspection with a hand lens. This process was time consuming, labor intensive, and had relatively little success. In the laboratory, detection of *A. auroguttatus* eggs oviposited onto bark pieces (approximately $12 \times 8$ cm) was also challenging due to the cryptic coloration and placement of the eggs deep inside crevices and cracks, and required a stereoscope and dissecting tools for identification. Since the overall structure (e.g., topography, thickness, and coloration) of oak bark from *A. auroguttatus* hosts makes surveying for egg parasitoids by collecting naturally deposited *A. auroguttatus* eggs an arduous task, our strategy of finding egg parasitoids using sentinel egg masses on filter paper is a practical though semi-artificial alternative.

The detection of *A. auroguttatus* egg parasitoids may also benefit from an increased search range and/or duration, especially in the native range in AZ. Our inability to detect *A. auroguttatus* egg parasitization in the introduced range of southern CA tentatively supports the enemy release hypothesis, and was not surprising, even considering the relatively short, eight week study period conducted from a single field site. Similarly, surveys for potential egg parasitoids of *A. planipennis* in its introduced range from 2003 to the present have yet to identify egg parasitoids that are indigenous to North America (Bauer et al., 2008; Liu et al., 2007). However, we did expect to detect higher rates of parasitization in the native range of *A. auroguttatus*. The very low percentage of *A. auroguttatus* parasitization in AZ (0.2%) is minute compared to the >60% parasitization of *A. planipennis* eggs reported from its native range in China (Liu et al., 2007), and could be the result of a more intensive search for *A. planipennis* egg parasitoids.

The *Trichogramma* sp. that was recovered from our study is not suitable for use in a classical biological control program against *A. auroguttatus* in CA due to its lack of host specificity, and its likely presence within infested areas in CA (this parasitoid has been previously recovered from desert areas in southern CA). The identification and utilization of egg parasitoids for the biological control of invasive wood borers such as *A. planipennis* and *Phoracantha semipunctata* (F.) (Coleoptera: Cerambycidae) have shown positive results (Duan et al., 2011; Hanks et al., 1996; Liu et al., 2007), and encourages further surveys for host specific egg parasitoids of *A. auroguttatus*. Specifically, we are interested in locating and identifying encyrtid parasitoids (Hymenoptera: Encyrtidae) of *A. auroguttatus*, should they exist. These parasitoids have been successfully used in the biological control of *A. planipennis*, *Agrilus anixius*, and *P. semipunctata*, and are known parasitoids of several other *Agrilus* species (Duan et al., 2012; Hanks et al., 1996; Muilenburg and Herms, 2012; Zhang et al., 2005). In addition, the highest rates of *Agrilus* egg parasitism (>50%) occurred with four species of encyrtid that were reported in North America, Asia, and Europe (Taylor et al., 2012). A comprehensive list of encyrtid egg parasitoids and their *Agrilus* hosts is presented in Taylor et al. (2012).

In order to maximize the potential detection of host specific egg parasitoids, and determine the potential impacts of natural ene-

mies attacking *A. auroguttatus* eggs in AZ and CA, a continuation of this study is needed across a larger number of field sites and over a longer period of time. Methods for conducting future egg parasitoid surveys will factor in results of this study which show non-cage and cage treatments as equally suitable for detecting and acquiring natural enemies of *A. auroguttatus* eggs.

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