An Investigation of the Coevolutionary Relationship Between *Aprostocetus hagenowii* (Ratzburg) (Hymenoptera: Eulophidae) and its Cockroach (Blattodea) Hosts

by

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Abstract

Oothecal parasitoid wasps (Hymenoptera) parasitize the egg cases (oothecae) of cockroaches (Blattodea). Oothecal parasitoids must have developed numerous adaptations to locate, parasitize, and develop within their hosts, but these adaptations have not been well studied. This goal of this dissertation was to investigate the coevolutionary relationships between oothecal parasitoids and their hosts primarily through the interactions of a generalist species *Aprostocetus hagenowii* (Ratzeburg)(Eulophidae) and its preferred host the American cockroach *Periplaneta americana* (L.) (Blattidae). During behavioral assays, gravid *P. americana* did not react to the presence of *A. hagenowii* or change their oviposition behavior. Additional experiments in total darkness or light, and electroantennogram assays indicate that *P. americana* does not react to or cannot detect *A. hagenowii* by sight or olfaction. No-choice assays were used to investigate the host range of *A. hagenowii*, and three new host species were recorded: *Blatta lateralis* (Walker) (Blattidae), *Neostylopyga propinqua* (Shelford) (Blattidae), and *Parcoblatta fulvescens* (Saussure and Zehntner) (Ectobiidae). Multi-generational no-choice assays were used to determine if *B. lateralis*, a peridomestic pest of growing concern, could support long-term populations of *A. hagenowii*. *Aprostocetus hagenowii* fitness rapidly declined with each generation of rearing on *B. lateralis* oothecae, which indicates challenges for the application of *A. hagenowii* for the biological control of *B. lateralis*. Lastly, the toxicity of baited insecticidal cockroach gels was compared between *A. hagenowii* and *P. americana*. Indoxacarb (Advion) caused non-significant ($P > 0.05$) *A. hagenowii* mortality but significant ($P < 0.01$) *P. americana* mortality, indicating its compatibility for use alongside *A. hagenowii*. Lack of correlation between the response of *A. hagenowii* and *P. americana* also indicates that there are differences in how the two species metabolize insecticides.

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List of Abbreviations

AAES	Alabama Agricultural Experiment Station
AOS	Advanced Onsite Sensor
CI	Confidence Interval
DCJW	*N*-decarbomethoxyllated JW062
EAG	Electroantennogram
EPS	Expanded Polystyrene
GC-MS	Gas Chromatography – Mass Spectrometry
IPM	Integrated Pest Management
L:D	Light:Dark
LT_{50}	Lethal Time (50%)
MST	Median Survival Time
Myr	Million Years
RH	Relative Humidity
SEM	Standard Error of the Mean
USDA	United States Department of Agriculture

Chapter 1: Introduction

Cockroaches are incredibly diverse numbering 4,600 species and inhabiting almost every habitat type (Roth and Willis, 1960; Bell et al., 2007). Reproductive features and modes are particularly important in the organization of cockroach phylogeny (McKittrick, 1964). The ancestral mode of reproduction among cockroaches, oviparity, involves enclosing the egg mass within a cuticle case (ootheca) and depositing it into the environment. There is typically little to no maternal care after the ootheca has been laid. A notable exception in maternal care among the oviparous cockroaches can be found in some Ectobiidae species, which retain the ootheca externally throughout its incubation (Roth, 1968, 1985). Only the family Blaberidae and four genera of Ectobiidae forgo oviparity in favor of an ovoviviparous mode (Roth, 1984, 1995). Ovoviviparous species build their ootheca externally, then retract it into a brood sac for incubation. Their oothecal cuticle is very thin to aid the transfer of water, gases, and some nutrients. Most nutrition is provided by the egg yolk (Bell et al., 2007). Viviparous blaberids are only known from a single genus, *Diploptera*, in which the developing embryos derive most of their nutrition through a milk-like secretion from the walls of the brood sac (Stay and Coop, 1974).

The reproductive modes and parental behaviors of cockroaches are of particular interest in the study of hymenopterous parasitoids using oothecae as hosts. The offspring of oviparous species are vulnerable to these wasps because they are left unguarded by parents. Past studies point to parasitoid wasps utilizing kairomones as the primary means of host location (Van Driesche and Hulbert, 1984; Suiter et al., 1996). Kairomones are pheromones (chemical messages) that benefit the receiver at the expense of the emitter, and they are used by most parasitoid wasp species to locate hosts (Vet and Dicke, 1992). There are multiple odor sources

available for oothecal parasitoids to exploit, including the ootheca's cuticle, the cement used to attach ootheca to substrates, and scents produced by cockroach nymphs and adults. Cockroaches and oothecal parasitoids have likely coexisted for hundreds of millions of years. Pressure from such parasitoids may have contributed to the switch from oviparous to ovoviviparous reproduction in cockroaches, and possibly led to the appearance of the Blaberidae about 130 Myr ago (McKittrick, 1964; Vishniakova, 1968; Wang, Z. et al., 2017). There are currently no known wasps specializing on the oothecae of ovoviviparous and viviparous species (LeBeck, 1991). Among oviparous species several strategies are employed to protect oothecae from attacks by parasitoid wasps and other organisms. One strategy is burial in soil, sand, or gravel (Figure 1.1). This behavior is the most common with examples in all oviparous families (McKittrick, 1964). Another method involves the use of secretions to attach oothecae to a substrate and cover it in debris. Burial and cementing behaviors are often both employed by the same species (McKittrick, 1964). If no suitable substrate is available for concealment, oothecae may be laid in aggregations (Figure 1.2) or simply dropped (Benson and Huber, 1989). Lastly, external retention of the ootheca is a third very rare strategy restricted to the Ectobiid genera of *Blattella*, *Chorisia*, and *Lophoblatta* (Roth, 1968, 1985). External retention is more resource and time intensive but allows the female to guard its ootheca from attacks throughout incubation (Bell et al., 2007).

Figures 1.1 and 1.2. (Left) A gravid *Neostylopyga propinqua* prepares a pit for oviposition. (Right) A group of *Supella longipalpa* oothecae cemented to canvas cloth. Note presence of the parasitoid *Comperia merceti* (circled in red).

How might parasitoids bypass the protections used by oviparous cockroach species? Past research indicates that kairomone-based cues are the main means of location for oothecal parasitoids, but research into the topic is largely limited to only two species of wasp, the eulophid *Aprostocetus hagenowii* (Ratzeburg) and the encyrtid *Comperia merceti* (Compere) (Van Driesche and Hulbert, 1984; Suiter et al., 1996). Even less work has focused on how the parasitoids overcome protective measures, such as burial, after they have located an ootheca. Bell et al. (2007) notes that parasitoid wasps may dig up buried oothecae but provides no further information. Is digging behavior common within the group? Do cockroaches change their oviposition behavior when parasitoids are detected nearby? How do their protective measures affect parasitoid host detection? The goal of this study is to gain a better understanding of the predator and prey dynamic between parasitoid wasps and their cockroach hosts.

Species Under Study - Cockroaches

Three cockroach families will be represented in this study: Blattidae, Ectobiidae, and Polyphagidae. The Blattid species include the American (*Periplaneta americana* (L.)), Oriental (*Blatta orientalis* L.), Turkestan (*Blatta lateralis* (Walker)), and African bullet (*Neostylopyga propinqua* (Shelford)) cockroaches. The American cockroach is one of the largest species under study and measures 29–53 mm in length. It is also one of the longest lived, with a lifespan of up to two years (Robinson, 2005). Characteristic of cockroaches, the egg case of the American cockroach is created by two colleterial glands located at the end of the abdomen (Brunet, 1951). These oothecae are dark brown to black and hold 14 to 16 eggs. Development time can vary greatly in response to temperature. Incubation at 30 °C leads to hatching after 24-38 days, while temperatures near 20 °C led to hatching after 74-92 days (Robinson, 2005). As a peridomestic pest species, the American cockroach can be found both indoors and outdoors where it lives and feeds among leaf litter, woodpiles, garbage dumps, and in the hollows of trees (Hagenbuch et al., 1988; Suiter et al., 1998). *Aprostocetus hagenowii* appears to prefer American cockroach oothecae and has been utilized for their control (Narasimham, 1984; Tee et al., 2011).

Blatta lateralis, the Turkestan cockroach, is a peridomestic pest cockroach that was introduced to the United States in the 1970s (Kim and Rust, 2013). Both the Turkestan cockroach and its congener the Oriental cockroach have similar lifespans. However, the Turkestan cockroach has faster development and greater fecundity, which have allowed its takeover of areas formerly dominated by Oriental cockroaches. Kim and Rust (2013) found that Turkestan cockroaches produce new oothecae every four to seven days requiring an average incubation period of about 40 days (26.7 ± 2°C). Conversely, the Oriental cockroach has a slower rate of oothecal production at 10 days on average and a longer incubation time (45-96

days at about 25 ± 2°C). Kim and Rust (2013) also note that the Turkestan cockroach was most likely introduced to the United States with military movements to and from its native range in the Middle East. The Turkestan cockroach's popularity as a feeder species for reptilian pets and ease of purchase on the internet will continue to facilitate its spread in North America (Kim and Rust, 2013). As a pest of growing concern, its suitability as a host for biocontrol agents, such as *A. hagenowii*, warrants investigation.

Neostylopyga propinqua (Figure 1.3), the African bullet cockroach, is a severely understudied species from tropical East Africa. This species is not classified as a pest and has been made globally available through the pet trade. Much of the information on its biology and behavior is only available through cockroach vendor webpages (e.g., Roachcrossing.com) as well as through the discussion boards of online forums such as Roachforum.com and Arachnoboards.com. *Neostylopyga propinqua* is about 20 mm long and produces an ootheca that is reddish-brown and holds up to 15 eggs. Incubation typically takes less than 30 days at 25 ± 2°C (Roachcrossing.com; Personal observations). This species is notable for producing a strong onion-like smell when disturbed (Personal observations).

Figure 1.3. An adult female *N. propinqua*. Their smaller size and red pronotal stripes help differentiate them from the other blattid species under study.

The first of the Ectobiidae to be examined is the brown-banded cockroach, *S. longipalpa* (Figure 1.4). Brown-banded cockroaches measure 11 to 14.5 mm long and live for about half a year. Rapid oothecal production makes up for their limited lifespan (Robinson, 2005). A new ootheca can be produced every six days, with an average of 11 oothecae over their lifespan (Cornwell, 1968). A genital secretion is used to attach the oothecae to a substrate, such as wood, cardboard, or upholstery (McKittrick, 1964). This facilitates their spread to new buildings and regions. Once inside a building, *S. longipalpa* is often found infesting a variety of areas as it does not require the high humidity preferred by many other cockroach pest species (Cornwell, 1968; Benson and Huber, 1989).

Figure 1.4. A mixed group of adult and nymph *S. longipalpa*. The canvas strips act as an easily movable ovipositional substrate, which are transferred to stinging cages holding *C. merceti* and *A. tenuipes*.

The German cockroach (*Blattella germanica* L.) could easily be considered the worst pest cockroach species owing in large part to the ease in establishment of infestations and the difficulty in their removal. They are cosmopolitan in distribution, and their spread has been facilitated by several factors: they are small (10 to 15 mm), nocturnal, develop quickly, produce many eggs per ootheca, and protect their oothecae by carrying them until they are ready to hatch (Robinson, 2005). The oothecae of German cockroaches can hold up to 32 eggs (Robinson, 2005). External retention of the ootheca increases offspring survival but also reduces reproductive output. Female German cockroaches can produce only four to nine oothecae over

their lifespan compared to the average of 11 made by *S. longipalpa* (Robinson, 2005; Bell et al., 2007).

Two species of wood cockroach, *Parcoblatta fulvescens* (Saussure and Zehntner) and *Parcoblatta lata* (Brunner von Wattenwyl), will be included in the study. Both species are native to the Southeastern US and are non-pests that prefer pineland, shrubland, and hammocks (Atkinson et al., 1991). *Parcoblatta fulvescens* is the smaller of the two, measuring up to 16.5 mm compared to just over 20 mm for *P. lata* (Blatchley, 1920). The oothecae of *P. fulvescens* are pill-like. In contrast, the oothecae of *P. lata* could best be described as having a banana-like shape due to their thin and slightly curved profile. Both species develop slowly and appear to be univoltine, at least when reared in culture. They typically bury their oothecae, which are highly susceptible to desiccation (Personal Observations and Discussions with Alan Jeon).

Two polyphagid species will be investigated. The Egyptian sand cockroach, *Polyphaga aegyptiaca* (L.), and Saussure's giant sand cockroach, *P. saussurei* (Dohrn) (Figures 1.5 and 1.6), are burrowing cockroaches ranging from the Mediterranean to the Middle East and North Africa (Grandcolas, 1996). They rarely act as peridomestic pests but otherwise lack economic importance (Robinson, 2005). A study by Farmani et al. (2019) suggests that *P. aegytiaca* and *P. saussurei* may be the same species despite their clear morphological differences. Similarly to *N. propinqua*, both species are poorly studied but have been made widely available through the pet trade, with online communication between enthusiasts being the primary source of information about their biology and behavior. *Polyphaga aegyptiaca* is the smaller of the two species at 30-34 mm for adult females compared to 35-44 mm for adult female *P. saussurei*. Both species produce an oothecae with a tab that the mother uses to maintain a grip on the case as it is towed

behind during burrowing (Figure 1.6) (Robinson, 2005; Roachcrossing.com; Personal Observations).

Figures 1.5 and 1.6. (Left) An adult female *P. saussurei* resting on author's hand. (Right) A *P. aegyptiaca* female clasping a newly formed ootheca by its tab.

Species Under Study - Wasps

There are three commercially available oothecal parasitoid species in the United States. These include the eupelmid *Anastatus tenuipes* Bolivar y Pieltain, *Aprostocetus hagenowii*, and *Comperia merceti* (Figures 1.7-1.9). *Aprostocetus hagenowii* is a generalist that is most often utilized in control of the American Cockroach, but it has at least eight other documented host species within the family Blattidae. *Anastatus tenuipes* and *C. merceti* are specialists on the brown-banded cockroach. However, there are limited reports that *A. tenuipes* can parasitize the egg cases of the German cockroach (LeBeck, 1991; Fallahzadeh et al., 2008). There are numerous other oothecal parasitoids that target cockroaches as hosts, and the evaniid wasp

Evania appendigaster (L.) is the most investigated due to its potential in biocontrol. Difficulties in maintaining other parasitoid species in culture and lack of efficient mass production have been an obstacle that prevents widespread availability and implementation (LeBeck, 1991; Tee and Lee, 2013).

Aprostocetus hagenowii is the most studied species of oothecal parasitoid, due to the high commercial value and importance of American cockroach control. It is gregarious, and groups of females will readily oviposit together into a single host despite the negative effects superparasitism has on their offspring (i.e. shorter lifespan, smaller body size, higher rate of mortality) (Narasimham, 1984). *Aprostocetus hagenowii* is the only species in which host location kairomones have been precisely identified. Suiter et al. (1996) found that the cuticular hydrocarbon 6,9-heptacosadiene, mucopolysaccharides from saliva, and calcium oxalate excreted by cockroach colleterial glands were used to locate hosts. They note that 6,9-heptacosadiene is found within the cuticle of the ootheca, and the other kairomones are deposited onto its surface during the process of oothecal formation and laying. The wide host range of *A. hagenowii* and, the relative ease in producing large numbers of female *A. hagenowii* led to this species becoming the focus of the research described in this dissertation.

Comperia merceti appears to use kairomones emitted from the adhesive that brown-banded cockroaches use to cement their oothecae in place; however, an analysis of its composition has yet to be undertaken (Van Driesche and Hulbert, 1984). *Anastatus tenuipes* is the least studied of these parasitoid species, and it is unknown what kairomones are used to find its host. Lastly, *Evania appendigaster*, which is not commercially available, has had very limited research conducted beyond the details of its development within its host. *Evania appendigaster* is relatively large, solitary, and produces only one offspring per ootheca (Tee and Lee, 2015).

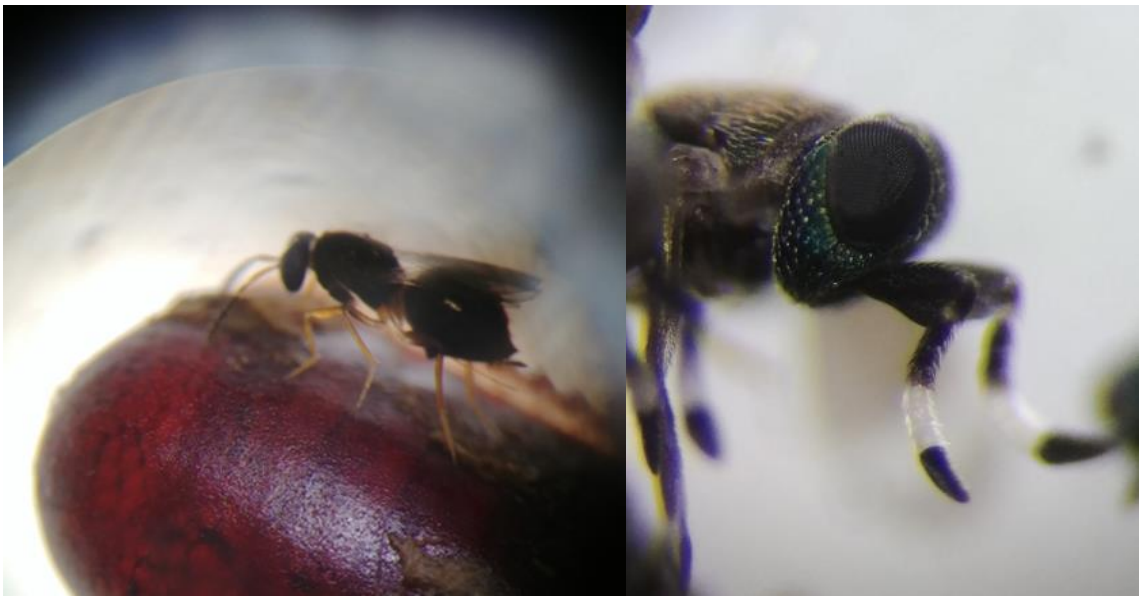

Figure 1.7 and Figure 1.8. (Left) A female *A. hagenowii* drums the surface of an American (*Periplaneta americana*) cockroach ootheca with its antennae. Antennal drumming is an early part of the behavioral sequence displayed during host assessment. (Right) A close-up view of the head and antennae of a female *C. merceti*. Females have three white antennal segments, while males have two at most.

Figure 1.9. A female *A. tenuipes* near one end of a *S. longipalpa* ootheca. Males are completely black in coloration, making for easy differentiation.

Insect Rearing

All cockroach colonies are provided with a similar diet of Purina Laboratory Diet 5001 rat chow blocks, Purina Dog Chow (Ralston Purina, St. Louis, MO), and/or carrots. Water is provided to all colonies via a jar with a wick. Food and water are replenished *ad libitum*. The *P. americana*, *B. lateralis*, and *S. longipalpa* colonies are reared in 2.0 gal plastic buckets. A 6.0 cm opening in the lid is screened with aluminum mesh and a layer of paper towel held in place by tape. A band of mineral oil near the top of the bucket helps to prevent escape while the lid is off during maintenance. The *P. americana* colony bucket has a ¼ inch (0.64 cm) hardware cloth screen at the bottom to separate the cockroaches from any debris, including oothecae, that fall into a second bucket below. A roll of hardware cloth is also used to provide harborage for both colonies. The brown-banded cockroach buckets have been outfitted with a wire holding thin strips of canvas to serve as harborage and oothecal-laying substrate.

The *N. propinqua*, *B. orientalis*, and *B. germanica* colonies are kept in glass jars sealed with one layer of paper towel and one layer of plastic screening held in place with rubber bands. Mineral oil near the jar's opening prevents escape. Corrugated cardboard tubes and sheets are provided as harborage. Both *Parcoblatta* and *Polyphaga* species are housed in plastic storage boxes with coconut fiber substrate (approx. 1.5 cm and 3 cm deep, respectively). The oothecae of *P. lata* and *P. fulvescens* are susceptible to low humidity requiring misting with water at least every other day. A lid helps to retain moisture while also preventing adult males from flying out, and mineral oil prevents climbing. Stacked sheets of corrugated cardboard provide harborage. Both polyphagids are burrowing, desert dwelling species that do not require high humidity levels to thrive. Their enclosures are lidded with a screen held in place with rubber bands. All cockroach colonies are kept in a room set to 27 ± 2 °C and a 14:10 (L:D) hour photoperiod.

Parasitized oothecae are kept in Petri dishes in an incubator set to 25 ± 2 °C and 15% RH. After emergence, a period of 24 hours must pass before the wasps are used in experiments so that mating can occur. Afterwards, oothecae from each wasp's preferred host are supplied using the following protocols. For *A. hagenowii*, females are placed into a Petri dish containing American cockroach ootheca between one to three weeks of age. Oothecae with damaged keels or dimples are avoided. For *A. tenuipes* and *C. merceti*, brown-banded oothecae are provided loose in Petri dishes and on hanging canvases in Plexiglas stinging cages. These cages are kept in the cockroach rearing room. *Anastatus tenuipes* requires oothecae that are less than two weeks old, while *C. merceti* is capable of successfully parasitizing oothecae up to three weeks old. Exposed oothecae are collected weekly and transferred to the incubator in Petri dishes. Once a colony of *Evania appendigaster* is established, the protocols for *A. hagenowii* will be followed. However, care will be taken to minimize disturbing parasitized ootheca as it has been reported that movement may kill the larvae (LeBeck, 1991).

Separating female wasps from males will be accomplished using their sexually dimorphic features. *Aprostocetus hagenowii* females have shorter antennal setae, which are considerably longer in males. *Anastatus tenuipes* females can be identified by their reddish-brown thorax and transparent first abdominal segment. Males are completely black in coloration. *Comperia merceti* females have three consecutive white antennal segments, and males have no more than two. Lastly, for *E. appendigaster*, the subtriangular shape of the female abdomen will be used to differentiate them from males, which have oval abdomens. Furthermore, in all four species females are typically larger than males. Females will be collected by an aspirator equipped with a pipette tip and a single ply of paper towel that prevents wasps from entering the aspirator's chamber.

Objectives

The first objective was to gain an understanding of the coevolutionary relationship between *A. hagenowii* and its cockroach hosts by examining the defensive behaviors of gravid cockroaches when confronted by parasitoids as well as how parasitoids bypass those defenses. The second objective was to explore and expand the known host range of *A. hagenowii* by including cockroach species often overlooked because they lack economic and medical importance. The third objective was to determine if *A. hagenowii* could maintain a long-term population using only the oothecae of the Turkestan cockroach (*B. lateralis*) and to determine if *A. hagenowii* could be used for biological control of the Turkestan cockroach. The final objective was to investigate how *A. hagenowii* responded to insecticides commonly applied as baited gels for control of peridomestic cockroaches.

References Cited

Atkinson TH, Koehler PG, Patterson RS. Catalog and atlas of the cockroaches (Dictyoptera) of North America North of Mexico. Misc. Publ. Entomol. Soc. Am. 1991:78:1–86.

Bell WJ, Roth LM, Nalepa CA. Cockroaches: ecology, behavior, and natural history. Baltimore (MD): The Johns Hopkins University Press; 2007.

Benson EP, Huber I. Oviposition behavior and site preference of the brownbanded cockroach, *Supella longipalpa* (F.) (Dictyoptera: Blattellidae). J. Entomol. Sci. 1989:24(1):84-91.

Brunet PCJ. The formation of the ootheca by *Periplaneta americana* I. The micro-anatomy and histology of the posterior part of the abdomen. Q. J. Microsc. Sci. 1951:92(18):113-127.

Cornwell PB. *The Cockroach: Volume 1*. Hutchinson and Co Ltd., London, UK 1968

Fallahzadeh M, Asadi R, Talebi AA. The first record of *Anastatus* (*Anastatus*) *tenuipes* (Hym.: Eupelmidae), a parasitoid of *Bellatella germanica* (Blattaria: Blattellidae) in Iran. J. Entomol. Soc. Iran. 2008:28(1):99-100.

Farmani M, Basseri H, Norouzi B, Gholizadeh S. Ribosomal DNA internal transcribed spacer 2 sequence analysis and phylogenetic comparison of seven cockroach species in northwestern Iran. BMC Res. Notes 2019:12:1-5.

Hagenbuch BE, Koehler PG, Patterson RS, Brenner RJ. 1988. Peridomestic cockroaches (Orthoptera: Blattidae) of Florida: their species composition and suppression. J. Med. Entomol. 1988:25(5):377-380.

Kim T, Rust MK. Life history and biology of the invasive Turkestan cockroach (Dictyoptera: Blattidae). J. Econ. Entomol. 2013:106(6):2428-2432.

LeBeck L. A review of the hymenopterous natural enemies of cockroaches with emphasis on biological control. Entomophaga 1991:36(3):335-352.

Narasimham AU. Comparative studies on *Tetrastichus hagenowii* (Ratzeburg) and *T. asthenogmus* (Waterston), two primary parasites of cockroach oothecae, and on their hyperparasite *Tetrastichus sp.* (*T. miser* (Nees) group) (Hymenoptera: Eulophidae). Bull. Entomol. Res.1984:74(2):175-189.

McKittrick FA. Evolutionary studies of cockroaches. Cornell University Agricultural Experiment Station Memoir, No. 389. Cornell University Press, Ithaca, NY. 1964.

Robinson WH. *Urban Insects and Arachnids: A Handbook of Urban Entomology*. Cambridge University Press, Cambridge UK. 2005.

Roth LM. *Pseudoanaplectinia yumotoi*, a new ovoviviparous myrmecophilous cockroach genus and species from Sarawak (Blattaria: Blattellidae; Blattellinae). Psyche 1995:102:79-87.

Roth LM. A taxonomic revision of the genus *Blattella* Caudell (Dictyoptera, Blattaria: Blattelidae). Entomol. Scand. Suppl. 1985:22:1-22.

Roth LM. *Stayella*, a new genus of ovoviviparous blattellid cockroach from Africa (Dictyoptera: Blattaria: Blattellidae). Entomol. Scand. 1984:15:113-139.

Roth LM. Oviposition behavior and water changes in the oothecae of *Lophoblatta brevis* Blatteria: Blattellidae: Plectopterinae. Psyche 1968:75:99-106.

Roth LM, Willis ER. The biotic associations of cockroaches. Smithsonian Misc. Collect. 1960:141(1):1-440.

Stay B, Coop AC. 'Milk' secretion for embryogenesis in a viviparous cockroach. Tissue Cell. 1974:6(4):669-693.

Suiter DR, Patterson RS, Koehler PC. Seasonal incidence and biological control potential of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) in treehole microhabitats. Environ. Entomol. 1998:27(2):434-442.

Suiter DR, Carlson DA, Patterson RS, Koehler PG. Host-location kairomone from *Periplaneta americana* (L.) for parasitoid *Aprostocetus hagenowii* (Ratzeburg). J. Chem. Ecol. 1996:22(4):637-651.

Tee H, Lee C. Water balance profiles, humidity preference and survival of two sympatric cockroach egg parasitoids *Evania appendigaster* and *Aprostocetus hagenowii* (Hymenoptera: Evaniidae; Eulophidae). J. Insect Physiol. 2015:77:45-54.

Tee HS, Saad AR, Lee CY. Evaluation of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) for the control of American cockroaches (Dictyoptera: Blattidae) in sewers and crevices around buildings. J. Econ. Entomol. 2011:104(6):2031-2038.

Van Driesche RG, Hulbert C. Host acceptance and discrimination by *Comperia merceti* (Compere) (Hymenoptera: Encyrtidae) and evidence for an optimal density range for resource utilization. J. Chem. Ecol. 1984:10:1399-1409.

Vet LEM, Dicke M. Ecology of infochemical use by natural enemies in a tritrophic context. Annu. Rev. Entomol. 1992:37:141-172.

Vishniakova VN. Mesozoic blattids with external ovipositors and peculiarities of their reproduction. *In* Jurassic Insects of Karatau. B. B. Rohdendorf, Ed. Akademiya Nauk SSSR, Ordelenie Obschej Biologii, Moscow, Russia. 1968.

Wang Z, Shi Y, Qiu Z, Che Y, Lo N. Reconstructing the phylogeny of Blattodea: robust support for interfamilial relationships and major clades. Sci. Rep. 2017:7:1-8.

Chapter 2: Cockroach oothecal laying behavior in response to the presence of parasitoid wasps

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Abstract

Several families of parasitic Hymenoptera have evolved traits that allow them to exploit cockroach oothecae. Cockroaches may bury and conceal their oothecae to prevent parasitoid attack. However, these protective measures require additional investment by females. We hypothesized that gravid cockroaches would reduce parental care in the absence of oothecal parasitoids and increase care when parasitoids were detected. Behavior bioassays consisted of glass jars containing a gravid American cockroach, *Periplaneta americana* (L.) (Blattodea: Blattidae), expanded polystyrene (EPS), and a dog food pellet. A fruit fly (*Drosophila melanogaster* Meigen) (Diptera: Drosophilidae) or parasitoid (*Aprostocetus hagenowii* (Ratzburg)) (Hymenoptera: Eulophidae) was added for the fly and parasitoid treatments, respectively. There was no significant difference among treatments in the proportion of oothecae buried or in mean cover of oothecae with EPS particles. Cover had no effect on parasitism success or failure. Electroantennogram (EAG) assays using *P. americana* antennae were also conducted. The EAG responses to dead parasitoid stimuli (0.111 to 0.124 mV) were significantly ($P < 0.05$) greater than the negative control, but responses to living parasitoid stimuli (0.075 to 0.089 mV) were non-significant. These findings suggest that burial and concealment of oothecae

is a general defensive behavior employed regardless of the presence or absence of a natural enemy. The results also indicate that gravid *P. americana* are unable to detect, and therefore, differentiate *A. hagenowii* from other insects and that *A. hagenowii* can successfully locate and parasitize oothecae completely concealed with EPS particles.

Introduction

The wasp *Aprostocetus hagenowii* (Ratzburg) (Hymenoptera: Eulophidae) is a generalist parasitoid of cockroach oothecae (egg cases) (LeBeck 1991). The American cockroach, *Periplaneta americana* (L.) (Blattodea: Blattidae) is its preferred host, but it has been recorded as a parasitoid of at least eight other cockroach species (LeBeck 1991). This parasitoid is small (≈ 2 mm), metallic-black in color, and has bright red eyes. Vinson and Piper (1986) and Suiter et al. (1996) found *A. hagenowii* were attracted to several kairomones emitted from *P. americana* and their oothecae. These kairomones include mucopolysaccharides in cockroach saliva and cuticular hydrocarbons from the cuticle of the ootheca. Gregarious, simple to rear, and using several pest cockroach species as hosts, *A. hagenowii* has been employed in programs aimed at urban cockroach control (LeBeck 1991, Tee et al. 2011). Much of the literature involving *A. hagenowii* has focused on its host finding and biocontrol efficacy, but little is known about how the hosts respond to the parasitoid's presence and attacks (Narasimham 1984, LeBeck 1991, Suiter et al. 1996, Tee et al. 2011).

Forming an ootheca requires female cockroaches to devote time, energy, and resources (water, protein, nitrogen, etc.) beyond the formation of the eggs themselves (Roth and Willis 1954b, Kramer et al. 1991). Oviparous cockroaches, such as *P. americana*, may expend additional resources towards the protection of their oothecae via burial, concealment, and/or

cementing in place at the oviposition site (McKittrick 1964, Schal et al. 1984). Previous studies by McKittrick et al. (1961), McKittrick (1964), and Yeh (1995) have found that substrate type, availability, and population density have major roles in determining when and how cockroaches employ defensive oviposition behaviors. McKittrick (1964) also found *Periplaneta spp*. displayed similar oviposition behaviors; however, *P. americana* tended to spend the most time and provide the most concealment compared to its congeners. One would expect the presence/absence of a natural enemy, such as an oothecal parasitoid, to play a role in cockroach oviposition behavior as well, but this has not been explored. Female *P. americana* frequently use saliva to cement their oothecae in place and attach concealing debris, but these secretions may make the oothecae more easily located by *A. hagenowii* (McKitterick 1964, Suiter et al. 1996).

Periplaneta americana is highly perceptive of odor, light, and air movement (Camhi and Tom 1978, Honkanen et al. 2015, Lockey and Willis 2015). Electroantennogram (EAG) studies exploring *P. americana* olfaction have largely focused on male cockroaches and their response to female sex pheromones (Norris and Chu 1974, Washio and Nishino 1976, Tsuchiya and Takahashi 1991, Lockey and Willis 2015). Notably, males can detect female sex pheromones in amounts as little as 0.1 nanograms (Tsuchiya and Takahashi 1991). Studies using female *P. americana* have found that both sexes often had similar electrophysiological responses to odors not associated with sex pheromones (Washio and Nishino 1976, Tsuchiya and Takahashi 1991).

Anecdotal observations of cockroaches held with other oothecal parasitoid wasps, such as *Anastatus floridanus* Roth and Willis (Hymenoptera: Eupelmidae) and *Evania appendigaster* (L.) (Hymenoptera: Evaniidae), showed that the cockroaches would kick and lunge at parasitoids that made physical contact with their bodies, oothecae, and oviposition sites (Roth and Willis 1954a, Yeh 1995). The aggressive behaviors displayed by the cockroaches indicates that they

recognize these parasitoid species as a danger to themselves or their offspring. We hypothesized that gravid cockroaches could distinguish between the presence and absence of both a harmless insect and a natural enemy, such as a parasitoid. A secondary goal was to examine how gravid *P. americana* may alter their parental care behaviors if a natural enemy is present at the time of oviposition. We hypothesized that a gravid cockroach would provide greater protection (burial or concealment) for its ootheca when parasitoids were present and less protection when parasitoids are absent. We used *P. americana*, *A. hagenowii*, and *Drosophila melanogaster* Meigen (Diptera: Drosophilidae), as the maternal host, natural enemy, and harmless insect, respectively, to test these hypotheses. Adult fruit flies were included in the study as they lack piercing mouthparts or other means to cause physical harm to cockroaches and their oothecae (Flatt 2020).

Methods and Materials

Insect Rearing

The *P. americana* used in this study came from colonies that have been reared at Auburn University since 1985 and have never been exposed to insecticides. Colonies were maintained in 121.1 L (32 gal) plastic garbage bins (Rubbermaid, Atlanta, GA) containing individuals of mixed sex and age. Purina Laboratory Diet 5001 rat chow blocks, Purina Dog Chow (Ralston Purina, St. Louis, MO), and water were provided *ad libitum*. Rolled and stapled corrugated cardboard tubes were provided as harborage. The cockroach colony room was maintained at 27 ± 2°C, 45–50% RH, and a 12:12 (L:D) photoperiod.

The *A. hagenowii* used in this study were received from Dr. Barry Pawson (PNE, Inc., Tipp City, OH; Retired) and have been reared at Auburn University since 2020. *Aprostocetus*

hagenowii were reared on oothecae of *P. americana* in 100 x 15 mm polystyrene Petri dishes (Thermo Fisher Scientific, Waltham, MA). Parasitoids were incubated within a growth chamber at 25 ± 2°C, 30–40% RH, and a 16:8 (L:D) photoperiod. *Aprostocetus hagenowii* will produce only male offspring if unmated (arrhenotokous), thus, a post emergence waiting period of 24 h was used before experiments to allow for mating. The *D. melanogaster* used in this study were of the "white" Canton-S (wCS) strain reared at Auburn University since 2018 (Beckmann et al. 2017). Larvae and adults were reared in cotton stoppered tubes containing a standard fruit fly diet (Archon Scientific, Durham, NC). The fruit fly colonies were maintained at 25 ± 2°C, 40–45% RH, and a 16:8 (L:D) photoperiod.

Behavioral Responses in the Presence or Absence of Parasitoids

Experimental units consisted of 0.47 L (1 pint) glass jars (Ball, Westminster, CO) containing a 1 x 4.5 x 10 cm plank of expanded polystyrene (EPS) (R-Tech Insulation Panel; Insulfoam, Puyallup, WA) as oviposition substrate. A band of petroleum jelly covering the inner opening of the jar prevented the escape of the cockroach. A fine mesh fabric secured over the jar opening with a rubber band prevented escape or entry of other insects. A single dog food pellet (Purina Dog Chow; Ralston Purina, St. Louis, MO) was included in each jar to reduce the incidence of oothecal cannibalism. The jars were arranged on a shelf as randomized blocks to limit possible confounding effects from position within the rearing room, which was maintained at 25 ± 2°C, 40 – 45% RH, and on a 16:8 L:D photoperiod. While observations made by Yeh (1995) indicate that *P. americana* will readily use EPS as a burial substrate for oothecae, no studies have quantified how well buried oothecae are concealed. Thus, 50 control replicates (gravid cockroach held alone) were conducted to determine baseline behavior.

Oviposition behavior experiments consisted of three treatments: a single gravid cockroach kept alone (control), with one *D. melanogaster* fly (fly treatment), or with one female *A. hagenowii* wasp (parasitoid treatment). Each experimental block consisted of 10 replicates per treatment and the experiment was repeated five times for a total of 50 replicates per treatment. *Periplaneta americana* cockroaches carrying mature oothecae (complete to near-complete cuticle tanning) were selected for experiments. All *A. hagenowii* parasitoids used were females aged at least 24 h post-emergence. The parasitoids had not been provided with fresh hosts prior to the experiment. The adult *D. melanogaster* selected for experiments were of random age and sex.

After 24 h, the cockroaches were removed from the jars, and the status of each ootheca (buried or dropped) was recorded. The concealment of each buried ootheca was quantified as estimated percent cover. Oothecae embedded in EPS without cover were given cover scores of 0%. Oothecae deposited outside of the provided substrate were considered dropped. If an ootheca was consumed by the cockroach, or if the cockroach died before oviposition could be attempted, it was excluded from analysis. Examples of cover estimation are shown in Fig. 2.1 a-c. The wasps used in the parasitoid treatment were left in their jars until their death (within 1 week) so that they could attempt to parasitize the oothecae without interference. Oothecae from the parasitoid treatment group were then incubated (about 1 month) under the same environmental conditions as the experiment and monitored for parasitoid emergence.

Additional Oviposition Experiments

The results of the initial oviposition behavior experiment were inconclusive (details provided in results). Thus, four additional experiments were devised to parse out the role density and vision

may have on *P. americana* detection and recognition of *A. hagenowii*. Parasitoid density experiments included gravid individuals of *P. americana* held alone (control), with 1 parasitoid, 2 parasitoids, 4 parasitoids, or 8 parasitoids (N = 4 per treatment). Replicate numbers are low because rearing a large number of *A. hagenowii* for what will be one-time use in a population density-based experiment is difficult. Their adult life span is short (≤ 1 wk) and many hundreds of parasitoids would be needed to have a replicate number comparable to that of the initial oviposition experiment. Similarly, fly density experiments were also carried out to compare the oviposition behaviors of *P. americana* held alone (control), with 1 fly, 2 flies, 3 flies, or 4 flies (N = 5 per treatment). Both parasitoid and fly density experiments were conducted using the same experimental unit set up, environmental conditions, and randomized block placement as described above.

A tertiary oviposition experiment involved holding gravid *P. americana* alone or with a single female *A. hagenowii* for 24 h in either dark or light conditions. Separate rooms were used so that treatments could be conducted simultaneously, and a red light was used to limit the chance that the cockroaches were able to see the parasitoids as they were added to the dark treatment jars (Song and Lee 2018). The red light was turned off after experimental set up was complete to achieve complete darkness. The experimental units, testing blocks, and environmental conditions of both rooms were as previously described.

The fourth experiment entailed observing gravid *P. americana* held with *A. hagenowii* in empty beakers (1.5 L). A band of petroleum jelly coating the upper wall of the beaker and paper towels held in place over the opening with rubber bands prevented escape. A cockroach was added to each beaker and kept alone until it displayed signs of calm (i.e., body still, limited antennal movement). A single female *A. hagenowii* was added to the first beaker, and ten

females were added to the second beaker. The cockroaches and parasitoids were monitored for at least 30 min and behavioral interactions were noted.

Electroantennogram

Electroantennogram (EAG) bioassays were conducted to determine if olfaction plays a primary role in *P. americana* detection and recognition of *A. hagenowii*. Assays were carried out with the Syntech® (Ockenfels Syntech GmbH, Buchenbach, Germany) CS 55 stimulus controller, IDAC-2 acquisition controller, and EAGPro software. A gravid *P. americana* was briefly (< 5 min) anesthetized over ice before a microblade was used to excise an antenna. Each antenna was excised between the pedicel and first flagellar segment. The distalmost four flagella were then excised. Electrode gel (SignaGel® Electrode Gel, Parker Laboratories, Fairfield, NJ) was used to attach the base and tip of the antenna to the reference and recording electrodes, respectively. Using work by Tsuchiya and Takahashi (1991) as a model, charcoal-filtered and humidified air (≈ 100% RH) was directed to flow over the upper half of the antennal preparation at 200 mL/min. Potential stimuli were placed in separate 2 mL glass Pasteur pipettes and each was injected into the continuous air stream as 0.2 sec puff of 25 mL/min flowing air.

Parasitoid stimuli tested included female *A. hagenowii* both dead (quantities of 1, 5, and 10 individuals) and living (quantities of 1 and 5). Fly stimuli also consisted of *D. melanogaster* of random sex, both dead and alive (both in quantities of 1 and 3 individuals). Fewer *D. melanogaster* were used to compensate for their larger size compared to *A. hagenowii*. Dead wasps and flies were obtained through natural mortality as they were held in Pasteur pipettes without food and water for several days. Testing of dead insect stimuli was carried out three days after death. Pieces of fine polyester mesh were packed above and below insects to hold them in

place within the pipettes. The control treatment consisted only of the mesh pieces within the pipette. An essential oil-based insecticide (Essentria© All Purpose Insect Concentrate, Envincio LLC, Cary, NC) was used as a positive control as it consistently generated antennal responses 4 – 9 times greater than the negative control. A single drop (≈ 50 μL) of the essential oil concentrate was added to a thin (≈ 5 x 60 mm) strip of filter paper (Whatman, Cytiva, Marlborough, MA), given 2 min to dry, then inserted into a pipette. For each antennal preparation the control was tested twice, and all other treatments were tested once with 1 min between treatments to allow the signal to return to baseline activity. The positive control was tested at regular intervals as the first, fifth, and tenth stimuli. In this type of experiment, low dose stimuli are typically tested first, and high doses last, to account for possible degradation of the antennal response over time (Roelofs 1984). However, testing of each antennal preparation took about 15 min and there was no noticeable degradation of response to the positive control. Thus, treatments were tested in randomized order regardless of dose. The maximum antennal depolarization (absolute mV) for each treatment per replicate was used for analysis.

Statistical Analysis

Data for estimated percent oothecal cover, parasitism success rate (i.e., proportion of parasitoid treatment oothecae that produced parasitoids compared to nymphs), ratio of dropped to buried oothecae, and EAG responses were not normally distributed and were therefore analyzed with non-parametric tests. Two-tailed Z-Tests were used for the buried/dropped proportional analysis. JASP software (Version 0.16.1; JASP Team 2022) was used to perform t-tests and Kruskal-Wallis ANOVAs with Dunn post-hoc analysis, where appropriate, for all other data. Statistical

significance was set to a threshold of $P < 0.05$ for all analyses. Data are described as means ± SEM (standard error of the mean).

Results

Behavioral Responses to the Presence or Absence of Parasitoids

In the baseline setting experiment, a majority (89.6%) of the cockroaches chose to bury their oothecae. The mean estimated cover for buried oothecae was 73.7 ± 0.4%. The primary oviposition experiment control group produced similar results: 91.8% buried, four oothecae dropped, and one cockroach dead before oviposition. The burial rate (89.8%) among cockroaches in the fly treatment group was also similar to that of the control ($z = 0.350$, $P = 0.726$). Four oothecae were dropped in the fly treatment, and one cockroach died before oviposition. The cockroaches in the parasitoid treatment had the lowest proportion (79.2%) of buried oothecae as there were twice as many dropped oothecae (10) compared to the other treatment groups. However, there was no significant difference between the parasitoid treatment's burial rate and that of any other group (control – parasitoid $z = 1.775$, $P = 0.075$; fly – parasitoid $z = 1.448$, $P = 0.147$). Two cockroaches in the parasitoid treatment died before oviposition.

The mean estimated cover of the buried oothecae in the fly (69.3 ± 0.4%) and parasitoid (73.7 ± 0.5%) treatments were not significantly different than that of the control (67.1 ± 0.4%) [Kruskal-Wallis ANOVA ($H(2) = 1.942$, $P = 0.379$)] (Fig. 2.2). Of the 48 oothecae laid in the parasitoid treatment group, 37.5% were parasitized, 47.9% produced nymphs, and 14.6% were nonviable (no emergence). Burial of oothecae with expanded polystyrene (EPS) particles provided no protection against parasitism as several of the oothecae completely (100%) covered

in EPS particles produced parasitoids. The mean estimated cover for the oothecae that were parasitized (74.6 ± 8.1%) was not significantly different from the cover provided to non-parasitized oothecae (75.5 ± 7.1%) [Mann-Whitney t-test (W = 140.50, P = 0.706)].

Additional Oviposition Experiments

All of the cockroaches in the parasitoid treatment groups buried their oothecae, while burial in the control group was 75%. Conversely, burial rate among cockroaches in the fly treatment groups ranged from 60% - 80%. Among the oothecae buried in the parasitoid experiment, estimated mean cover remained consistently high (> 80.0%), except in the treatment containing four parasitoids (75.0 ± 0.5%). Concealment of oothecae in the fly experiment fell, then rose with increasing fly density: control = 80.0 ± 1.4%, 1 fly = 62.5 ± 1.5%, 2 flies = 60.0 ± 2.5%, 3 flies = 80.0 ± 1.0%, and 4 flies = 95.0 ± 0.5%.

In the light-dark experiment, the proportion of cockroaches that buried their oothecae was similar among groups: control-light = 93.3%, control-dark = 86.2%, parasitoid-light = 89.6%, and parasitoid-dark = 86.2% (Two-tailed Z-Test, all combinations $P \geq 0.05$). Mean estimated cover of buried oothecae was also similar in both light (control = 77.9 ± 4.2%; parasitoid = 78.1 ± 4.8%) and dark treatment groups (Control = 72.8 ± 5.7%; parasitoid = 76.5 ± 5.2%) [Kruskal-Wallis ANOVA (H)(3) = 0.644, P = 0.886)].

Electroantennogram

Seventeen replicates using dead insects as stimuli, and six replicates utilizing living insect stimuli were analyzed. EAG responses to dead insect stimuli were numerically higher overall than the responses recorded from the testing of living insects, but the difference was non-

significant [Welch's t-test ($t(128.5) = 1.883$, $P = 0.062$)]. The EAG responses to dead insects were: mean negative control (air only) 0.073 ± 0.013 mV, mean positive control (essential oil) 0.966 ± 0.068 mV, 1 parasitoid = 0.111 ± 0.016 mV, 5 parasitoids = 0.124 ± 0.017 mV, 10 parasitoids = 0.116 ± 0.015 mV, 1 fly = 0.095 ± 0.015, and 3 flies = 0.103 ± 0.014. The responses to the positive control and parasitoid treatment stimuli were significantly higher compared to the responses to the negative control and fly treatment stimuli [Kruskal-Wallis ANOVA ($H(6) = 49.452$, $P < 0.0001$) (Fig. 2.3) and Dunn's post-hoc (Table 2.1)].

Among the antennae used to test living insect stimuli, the response to the mean negative control (air only) was higher (0.104 ± 0.012 mV) compared to the mean negative control in the dead insect experiment. Yet, the overall responses to stimuli were lower: mean positive control (EO) 0.460 ± 0.053, 1 parasitoid 0.75 ± 0.008, 5 parasitoids 0.089 ± 0.011, 1 fly 0.075 ± 0.012, and 3 flies 0.118 ± 0.014. Only the positive control produced results significantly higher than the negative control [Kruskal-Wallis ANOVA ($H(5) = 20.414$, $P < 0.0001$) (Fig. 2.4) and Dunn's post-hoc (Table 2.2)].

Discussion

The results of the oviposition behavior and electroantennogram (EAG) experiments provide no conclusive evidence that *P. americana* can detect *A. hagenowii* via sight or scent. At the outset of this study, we expected that gravid cockroaches would apply less defensive oviposition behaviors (dropping of the ootheca) when isolated and more defensive behaviors (burial and concealment of oothecae) when a parasitoid was present. Instead, we found no significant difference in oviposition behavior between cockroaches held alone, with *D. melanogaster*, or with *A. hagenowii*. The cockroaches in the parasitoid treatment that buried their oothecae did not

provide significantly greater cover, and what cover was provided made no difference in preventing parasitoid attack. We also expected antennal responses to both parasitoid and fly stimuli to be greater than the negative control (air), but only the dead parasitoid treatments elicited significant responses.

We describe two possible explanations why gravid *P. americana* cockroaches in the present study failed to differentiate the presence of living *A. hagenowii* from other insects or take effective action to protect their oothecae. First, the morphology and behavior of *A. hagenowii* may be adapted to aid in minimizing detection or recognition by hosts. *Aprostocetus hagenowii* is a very small wasp (≈ 2 mm), and anecdotal observations of their behaviors throughout this study suggest that much of their time is spent either stationary or slowly wandering. A minute object with little movement may go unnoticed by the much larger cockroach. Furthermore, the quantity of odor released by an insect is likely to be proportional to its body's surface area (Cardé and Willis 2008). The EAG treatments containing a single parasitoid elicited a lower antennal response than those containing five or ten. Such responses were made with the EAG apparatus delivering odor plumes directly to antennae. The cockroaches used for the oviposition experiments may have been less likely to detect the odors of a single parasitoid within their jar because the mesh cap did not prevent the intrusion of odors from the surrounding area. Yeh's (1995) description of interactions between *P. americana* and *E. appendigaster* provides anecdotal evidence that mother cockroaches will respond aggressively (i.e., lunge) towards this species of oothecal parasitoid if it approaches the burial site of an ootheca. We observed no such behavior between *P. americana* and *A. hagenowii*. Instead, the gravid cockroaches only reacted to parasitoids that made repeated physical contact with their body. These interactions were observed rarely and were ended by a kick or a flick of the antenna from the cockroach. Such

physical interactions between *P. americana* and *A. hagenowii* are unlikely to occur naturally except under high density conditions in enclosed spaces. Thus, the responses of the *P. americana* held in the beakers are probably not specific to an encounter with *A. hagenowii*. A key difference between *A. hagenowii* (≈ 2 mm) and *E. appendigaster* (≈10 mm) is the latter's relatively large size, which may render this species more noticeable to *P. americana* (Smith et al. 2022).

Second, several studies examining the behavior of *P. americana* in the presence of predators suggest this species has poor predator awareness and avoidance behaviors (Stierle et al. 1994, Comer et al. 1994, Catania 2018). Studies by Stierle et al. (1994) and Comer et al. (1994) found that adult *P. americana* typically initiated escape or defense behaviors after a predator, such as a spider or mouse, made its attack. In some cases, the cockroaches began their escape only after the attacker made physical contact. During the study by Catania (2018), about half of the cockroaches held with emerald jewel wasps, *Ampulex compressa* (Fabricius) (Hymenoptera: Ampulicidae) failed to detect the presence of the wasps, making no initial attempts at defense as they were attacked. These studies suggest that *P. americana* rely almost entirely on physical contact and wind movement cues via their legs, antennae, and cerci to detect and respond to enemies. Among the predator species examined, *A. compressa* was the smallest and typically approached prey on foot, rather than by flight, two factors that may have played key roles in its ability to avoid detection (Catania 2018). The small size and discreet movements of *A. hagenowii* likely make adult *P. americana* similarly blind to its presence. The responses of *P. americana* antennae to the dead, rather than living, insects may be due to this species' nature as a detritivore (Roth and Willis 1957). *Periplaneta americana* has been observed to consume the carcasses of dead insects, and its antennae may be more sensitive to the odor of dead insects if such scents are associated with a potential food source (Roth and Willis 1957, Bell and Adiyodi 1981).

Aprostocetus hagenowii lives in close association with cockroach oothecae, while the *D. melanogaster* reared for this study lived in a relatively clean and controlled environment. Such differences may have played a role in the quality of their odor after their death as well as the greater antennal response of *P. americana* towards dead parasitoid stimuli. *Periplaneta americana* must contend with a diverse range of natural enemies and appears to utilize a general suite of escape and defense behaviors to thwart most attackers, including conspecifics (Bell and Sams 1973, Comer et al. 1994, Catania 2018). Burial and concealment of oothecae, even when the mother is isolated, may also be a general defensive behavior adopted to protect offspring from attack (McKittrick 1964). Against enemies that are minute in size and/or lack digging ability, such as *A. hagenowii*, burial deep within a substrate would likely prevent attack. Anecdotal observations of oothecae deeply buried in sand and ground coconut fiber found that *A. hagenowii* could often locate the burial location but were unable to move the overlying substrate to gain access for oviposition. The oothecae well concealed by EPS particles in the oviposition experiments were not deeply buried, thus, *A. hagenowii* was probably able to reach the oothecal cuticle through small gaps or by piercing the thinly applied layer of cover. Observations made by Yeh (1995) of *E. appendigaster* removing EPS particles to access buried oothecae suggests that larger predators are capable of moving smaller particles used for concealment. Given that *P. americana* readily oviposits in EPS, which is commonly used as a packing, storage, and insulation material in homes and businesses, the ability of both *A. hagenowii* and *E. appendigaster* to locate and parasitize oothecae hidden in this substrate indicates that their efficacy as biocontrol agents will not be significantly impeded by this material.

The *P. americana* used during this study have been reared under laboratory conditions for several decades, and presumably only risk attacks and aggressive encounters from conspecific

colony members. Long-term rearing of cockroaches and other insects under laboratory conditions can lead to loss of the genetic diversity and behavioral characteristics typical of wild populations (Huettel 1976, Akers and Robinson 1983). Cockroaches from wild populations may respond to natural enemies in a manner different from what is commonly observed in laboratory strains. While *P. americana* tend to be nocturnal, those from this study's laboratory colonies will carry out foraging, mating, and oviposition behaviors under lighted conditions (Bell and Adiyodi 1981, Personal Observations). Wild and laboratory reared *P. americana* might differ in their behavior under light, but further study is needed to understand these differences. *Aprostocetus hagenowii* is diurnal and phototactic but can successfully locate and parasitize oothecae held in dark spaces (i.e., sewers, cabinets, etc.) preferred by their hosts (Tee et al 2011). A female *P. americana* ovipositing during the daytime or in a well-lit room may be more likely to encounter diurnal parasitoids, which may increase the chance that the ootheca is located and parasitized. However, the role that light plays in cockroach-oothecal parasitoid interaction has yet to be investigated.

Of future interest is how other cockroach species and their natural enemies interact when encountering each other near ootheca oviposition sites. If the small size and discreet behavior of *A. hagenowii* are the primary factors allowing it to evade detection or recognition by *P. americana*, one would expect that the much larger *E. appendigaster* is less likely to go unnoticed. A comparison of behavior between laboratory and wild strains of cockroaches is also warranted.

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References Cited

Akers RC, Robinson WH. Comparison of movement behavior of three strains of German cockroach, *Blattella germanica*. Entomol. Exp. Appl. 1983:34(2):143-147.

Beckmann JF, Ronau JA, Hochstrasser MA. A *Wolbachia* deubiquitylating enzyme induces cytoplasmic incompatibility. Nat. Microbiol. 20172: 1-7.

Bell WJ, Adiyodi KG. The American Cockroach. Chapman and Hall, New York, NY. 1981.

Bell WJ, Sams GR. Aggressiveness in the cockroach *Periplaneta americana* (Orthoptera, Blattidae). Behav. Biol. 1973:9(5):581-593.

Camhi JM, Tom W. The escape behavior of the cockroach *Periplaneta americana* – I: Turning response to wind puffs. J. Comp. Physiol. 1978:128: 193-201.

Cardé RT, Willis MA. Navigational strategies used by insects to find distant, wind-borne sources of odor. J. Chem. Ecol. 2008:34(7):854-866.

Catania KC. How not to be turned into a zombie. Brain Behav. Evol. 2018:92(1-2):32-46.

Comer CM, Mara E, Murphy KA, Getman M, Mungy MC. Multisensory control of escape in the cockroach *Periplaneta americana* – II. Patterns of touch-evoked behavior. J. Comp. Physiol. A 1994:174:13-26.

Flatt T. Life-history evolution and the genetics of fitness components in *Drosophila melanogaster*. Genetics 2020:214:3-48.

Goss-Sampson MA. Statistical Analysis in JASP 0.12: A Guide for Students. University of Greenwich, London, UK. 2020.

Honkanen A, Takalo J, Heimonen K, Vähäsöyrinki M, Weckström M. Cockroach optomotor responses below single photon level. J. Exp. Biol. 2014:217(23):4262-4268.

Huettel MD. Monitoring the quality of laboratory-reared insects: a biological and behavioral perspective. Environ. Entomol. 1976:5(5):807-814.

Kramer KJ, Christensen AM, Morgan TD, Schaefer J, Czapla TH, Hopkins TL. Analysis of cockroach oothecae and exuviae by solid state ^{13}C-NMR spectroscopy. Insect Biochem. 1991:21(2):149-156.

LeBeck LM. A review of the hymenopterous natural enemies of cockroaches with emphasis on biological control. Entomophaga 1991:36:335-352.

Lockey JK, Willis M. One antenna, two antennae, big antennae, small: total antennae length, not bilateral symmetry, predicts odor-tracking performance in the American cockroach *Periplaneta americana*. J. Exp. Biol. 2015:218(14):2156-2165.

McKittrick FA. Evolutionary studies of cockroaches. Cornell University Agricultural Experiment Station Memoir, No. 389. Cornell University Press, Ithica, NY.1964.

McKittrick FA., Eisner T, Evans HE. Mechanics of species survival. J. Nat. Hist. 1961:70: 46-50.

Narasimham AU. Comparative studies on *Tetrastichus hagenowii* (Ratzeburg) and *T. asthenogmus* (Waterston), two primary parasites of cockroach oothecae, and on their

hyperparasite *Tetrastichus sp.* (*T. miser* (Nees)group) (Hymenoptera: Eulophidae). Bull. Entomol. Res.1984:74(2):175-189.

Norris DM, Chu H. Chemosensory mechanism in *Periplaneta americana*: electroantennogram comparisons of certain quinone feeding inhibitors. J. Insect Physiol. 1974:20(9):1687-1696.

Roelofs WL. Electroantennogram assay: rapid and convenient screening procedures for pheromones. In H.E. Hummel and T.A. Miller (eds.), Techniques in Pheromone Research. Springer-Verlag, New York, NY. 1984.131–160.

Roth LM, Willis ER. *Anastatus floridanus* (Hymenoptera: Eupelmidae) a new parasite on the eggs of the cockroach *Eurycotis floridana*. Trans. Am. Entomol. Soc. 1954a:80(1):29-41.

Roth LM, Willis ER. The reproduction of cockroaches. Smithsonian Miscellaneous Collections 1954b:122:1-49.

Roth LM, Willis ER. The medical and veterinary importance of cockroaches. Smithsonian Miscellaneous Collections 1957:134:1-137.

Schal C, Gautier JY, Bell WJ. Behavioral ecology of cockroaches. Biol. Rev. 1984:59(2):209-254.

Smith CM, Appel AG, Hu XP. Cockroach biocontrol: parasitoids and parasites. Alabama Cooperative Extension System. 2022. 1-4.

Song BM, Lee CH. Toward a mechanistic understanding of color vision in insects. Front. Neural Circuits. 2018:12:1-9.

Stierle IE, Getman M, Comer CM. Multisensory control of escape in the cockroach *Periplaneta americana* – I. Initial evidence from patterns of wind-evoked behavior. J. Comp. Physiol. A 1994:174:1-11.

Suiter DR, Carlson DA, Patterson RE, Koehler PG. Host-location kairomone from *Periplaneta americana* (L.) for parasitoid *Aprostocetus hagenowii* (Ratzeburg). J. Chem. Ecol. 1996:22:637-651.

Tee H, Saad AR, Lee CY. Evaluation of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) for the control of American cockroaches (Dictyoptera: Blattidae) in sewers and crevices around buildings. J. Econ. Ent. 2011:104(6):2031-2038.

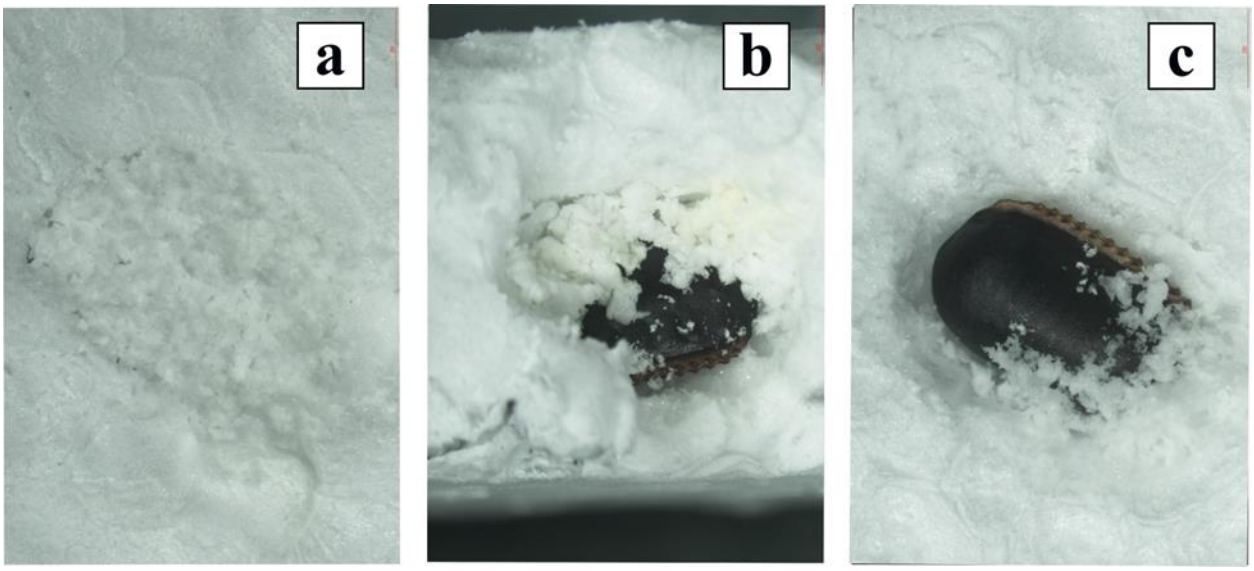
Tsuchiya T, Takahashi S. Electroantennogram (EAG) response to synthetic periplanones in *Periplaneta* and *Blatta* cockroaches. Appl. Ent. Zool. 1991:26(3):327-333.

Vinson SB, Piper GL. Source and characterization of host recognition kairomones of *Tetrastichus hagenowii*, a parasitoid of cockroach eggs. Physiol. Entomol. 1986:11(4):459-468.

Washio H, Nishino C. Electroantennogram responses to the sex pheromone and other odours in the American cockroach. J. Insect Physiol. 1976:22(5):735-741.

Yeh C. Oviposition concealment behavior of *Periplaneta americana* (L.) and its application on oothecal trap in the laboratory. Formosan Entomol. 1995:15:153-161.

Figures and Tables



Figs. 2.1a-c. a) displays an ootheca with 100% estimated cover, b) provides an example of an ootheca with about 60% cover**,** and c) is an example of an ootheca with about 30% cover with EPS particles.

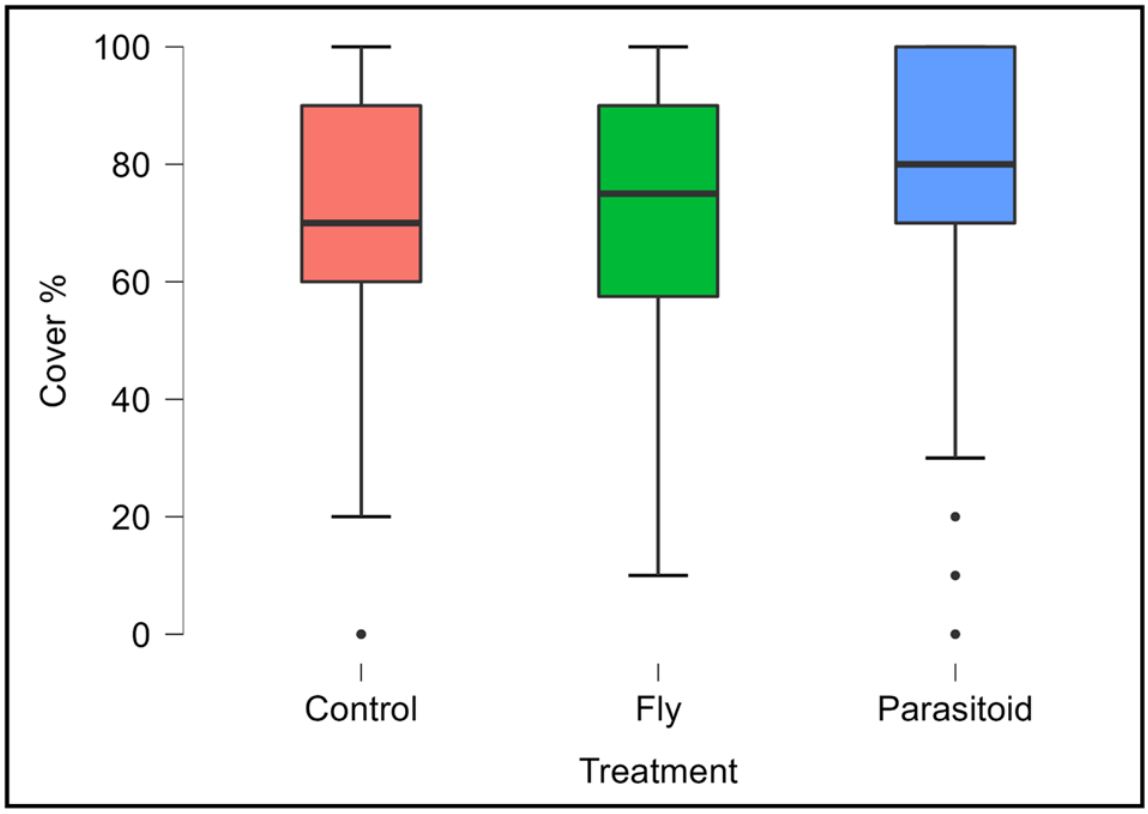


Fig. 2.2. Comparison of mean estimated percent cover of oothecae between treatments in the primary oviposition experiment.

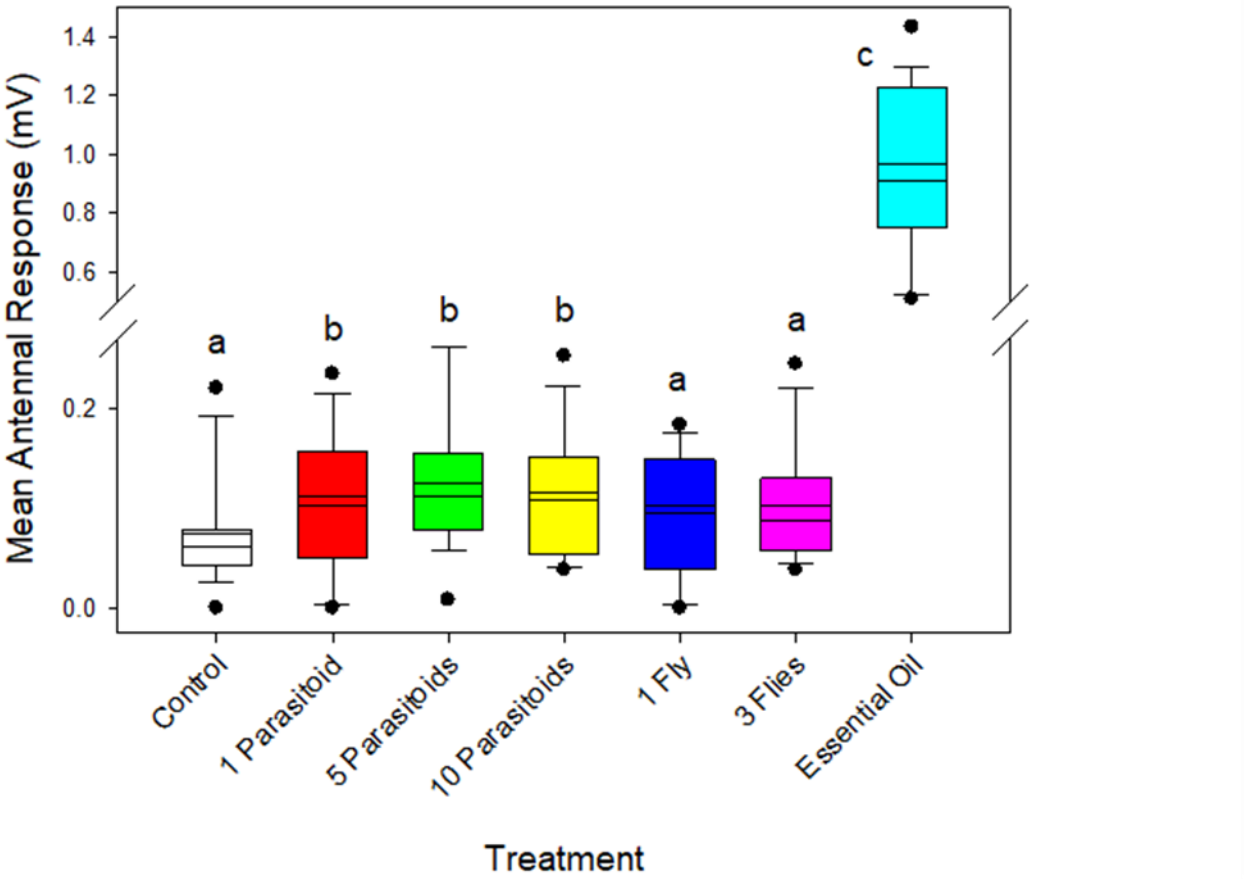


Fig. 2.3. Comparison of *Periplaneta americana* antennal responses to dead insect stimuli. The *A. hagenowii* parasitoid treatments elicited a significantly greater response (all $P < 0.05$) compared to the control. The positive control (essential oil) elicited significantly higher results compared to all other treatments ($P < 0.0001$). Different letters denote significant differences between groups ($P \leq 0.05$).

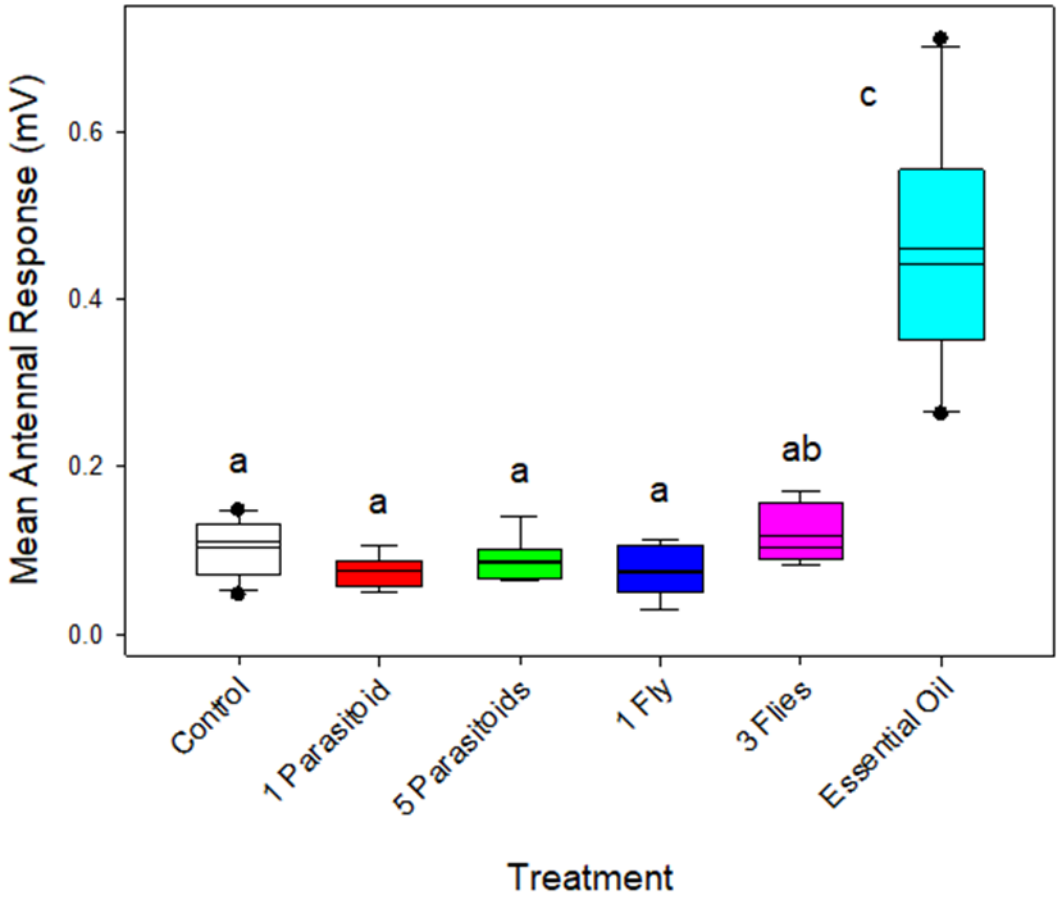


Fig. 2.4. Comparison of *Periplaneta americana* antennal responses to living insect stimuli. Only the positive control (EO) elicited a significantly greater response than the control ($P = 0.008$). Antennal response to the 3 flies treatment was significantly greater than that of the 1 parasitoid and 1 fly treatments. Different letters denote significant differences between groups ($P \leq 0.05$).

Table 2.1. Results of Kruskal-Wallis ANOVA (H(6) = 49.452, $P < 0.0001$) with Dunn's post hoc test comparing maximum antennal response (mV) between dead insect stimuli. Significant values ($P < 0.05$) are in bold font.

Dunn's Post Hoc Comparisons of EAG Responses to Dead Insect Stimuli

Treatment	(-) Control	1 Parasitoid	5 Parasitoid	10 Parasitoid	1 Fly	3 Flies	(+) Control
(-) Control	-	z = -1.775, ***P* = 0.038**	z = -2.247, ***P* = 0.012**	z = -1.872, ***P* = 0.031**	z = -1.343, *P* = 0.090	z = -1.340, *P* = 0.090	z = -6.459, ***P* < 0.0001**
1 Parasitoid	z = -1.775, ***P* = 0.038**	-	z = -0.472, *P* = 0.318	z = -0.097, *P* = 0.461	z = 0.433, *P* = 0.333	z = 0.435, *P* = 0.332	z = -4.684, ***P* < 0.0001**
5 Parasitoid	z = -2.247, ***P* = 0.012**	z = -0.472, *P* = 0.318	-	z = 0.375, *P* = 0.354	z = 0.905, *P* = 0.183	z = 0.907, *P* = 0.182	z = -4.212, ***P* < 0.0001**
10 Parasitoid	z = -1.872, ***P* = 0.031**	z = -0.097, *P* = 0.461	z = 0.375, *P* = 0.354	-	z = 0.530, *P* = 0.298	z = 0.532, *P* = 0.297	z = -4.587, ***P* < 0.0001**
1 Fly	z = -1.343, *P* = 0.090	z = 0.433, *P* = 0.333	z = 0.905, *P* = 0.183	z = 0.530, *P* = 0.298	-	z = 0.002, *P* = 0.499	z = -5.117, ***P* < 0.0001**
3 Flies	z = -1.340, *P* = 0.090	z = 0.435, *P* = 0.332	z = 0.907, *P* = 0.182	z = 0.532, *P* = 0.297	z = 0.002, *P* = 0.499	-	z = -5.119, ***P* < 0.0001**
(+) Control	z = -6.459, ***P* < 0.0001**	z = -4.684, ***P* < 0.0001**	z = -4.212, ***P* < 0.0001**	z = -4.587, ***P* < 0.0001**	z = -5.117, ***P* < 0.0001**	z = -5.119, ***P* < 0.0001**	-

Table 2.2. Results of Kruskal-Wallis ANOVA (H(5) = 20.414, p < 0.0001) with Dunn's post hoc test comparing maximum antennal response (mV) between living insect stimuli. Significant values ($p < 0.05$) are in bold font.

Dunn's Post Hoc Comparisons of EAG Responses to Living Insect Stimuli

Treatment	(-) Control	1 Parasitoid	5 Parasitoids	1 Fly	3 Flies	(+) Control
(-) Control	-	z = 1.357, *P* = 0.087	z = 0.740, *P* = 0.230	z = 1.274, *P* = 0.101	z = -0.630, *P* = 0.264	z = -2.412, ***P* = 0.008**
1 Parasitoid	z = 1.357, *P* = 0.087	-	z = -0.617, *P* = 0.269	z = -0.082, *P* = 0.467	z = -1.987, ***P* = 0.023**	z = -3.768, ***P* < 0.0001**
5 Parasitoids	z = 0.740, *P* = 0.230	z = -0.617, *P* = 0.269	-	z = 0.534, *P* = 0.297	z = -1.370, *p* = 0.085	z = -3.152, ***P* < 0.0001**
1 Fly	z = 1.274, *P* = 0.101	z = -0.082, *P* = 0.467	z = 0.534, *P* = 0.297	-	z = -1.905, ***P* = 0.028**	z = -3.686, ***P* < 0.0001**
3 Flies	z = -0.630, *P* = 0.264	z = -1.987, ***P* = 0.023**	z = -1.370, *P* = 0.085	z = -1.905, ***P* = 0.028**	-	z = -1.781, ***P* < 0.0001**
(+) Control	z = -2.412, ***P* = 0.008**	z = -3.768, ***P* < 0.0001**	z = -3.152, ***P* < 0.0001**	z = -3.686, ***P* < 0.0001**	z = -1.781, ***P* < 0.0001**	-

Chapter 3: Host range of the oothecal parasitoid *Aprostocetus hagenowii* (Hymenoptera: Eulophidae)

Abstract

Aprostocetus hagenowii is a generalist parasitoid of cockroach (Blattodea) oothecae. Previous studies examining the host range of *A. hagenowii* have largely focused on cockroaches of economic and medical importance, which represent a minority of species in an order filled with species of diverse morphology, behavior, and ecology. The aim of this study was to expand the known host range of *A. hagenowii* with emphasis on non-pest as well as pest species from three cockroach families (Blattidae, Corydiidae, and Ectobiidae). Previously recorded host species were also reexamined. Oothecae from 17 cockroach species were exposed to *A. hagenowii*. Three new host species were recorded: *Blatta lateralis* (Walker) (Blattidae), *Neostylopyga propinqua* (Shelford) (Blattidae), and *Parcoblatta fulvescens* (Saussure and Zehntner) (Ectobiidae). Among the reexamined host species *Periplaneta australasiae* (Fab.) (Blattidae), *Blatta orientalis* L. (Blattidae), and *Neostylopyga rhombifolia* (Stoll) (Blattidae) were successfully parasitized. The cuticle thicknesses of 7 cockroach species' oothecae were also investigated. There were significant differences [Kruskal-Wallis ANOVA: all zones (below keel, side, and bottom) measured $P < 0.001$] in cuticle thickness among the species measured. *Polyphaga sassurei* (Dohrn)(Corydiidae) and *Eurycotis floridana* (Walker) (Blattidae) had the thickest cuticles (all zones > 0.09 mm) and *Blattella germanica* (L.) (Ectobiidae) had the thinnest (all zones < 0.03 mm). However, mean *A. hagenowii* ovipositor length (0.92 ± 0.012 mm) far

exceeded the thickest oothecae measured. Oothecal cuticle thickness alone was not observed to determine host suitability of each tested cockroach species for *A. hagenowii*.

Introduction

Aprostocetus hagenowii (Ratzeburg) is a generalist parasitoid wasp that parasitizes the oothecae of cockroaches (Blattodea) (LeBeck 1991). Most known hosts are peridomestic pest species in the family Blattidae, such as the American cockroach *Periplaneta americana* (L.), the Oriental cockroach *Blatta orientalis* L., the harlequin cockroach *Neostylopyga rhombifolia* (Stoll), and the Florida woods cockroach *Eurycotis floridana* (Walker) (LeBeck 1991). An unknown species of wood cockroach in the genus *Parcoblatta* (Ectobiidae) was also reported as a host for *A. hagenowii* (Edmunds 1952). The German cockroach *Blattella germanica* (L.) (Ectobiidae) has been listed as a host as well, but the current consensus is that these reports are erroneous (Roth and Willis 1960, LeBeck 1991).

Investigations of parasitoid host ranges are often conducted in relation to species of importance from the human perspective – to find new pest host species and to determine what non-target organisms would be at risk when the parasitoid is released for pest control (Van Driesche 2004). Past investigations of *A. hagenowii* host range have followed this pattern (Roth and Willis 1954, Roth and Willis 1960, Narasimham and Sankaran 1979, Harlan and Kramer 1980, Suiter et al. 1996). This focus on cockroaches of human importance has heavily biased studies of *A. hagenowii* biology towards a minority of cockroach species in an order filled with diverse morphologies, habitats, and behaviors (Roth and Willis 1960, Bell et al. 2007).

Inclusion of non-pest insect species in parasitoid host range studies has several obstacles. Funding focused on the ecology, biology, and natural history of non-pest insect species is limited (Slade and Ong 2023). Exotic non-pest species may be harder to obtain, especially if they are native to a country or region outside of the researcher's own, and importation of exotic organisms typically requires a permitting process through governmental entities, such as the United States Department of Agriculture (USDA): Animal and Plant Health Inspection Service (APHIS). Furthermore, these species may lack established rearing techniques or may be adapted to environments that are difficult to replicate in a laboratory setting.

Over the past two decades the proliferation of hobbyists involved in the online discussion and trade of exotic arthropods has made it easier for researchers to obtain non-pest species as well as instruction on their rearing. Easier access to exotic and non-pest cockroach species provides an opportunity for a more thorough investigation of the host range and coevolutionary relationship between *A. hagenowii* and cockroach oothecae. Testing of cockroach species that share their native ranges with *A. hagenowii* would be a priority. However, the exact native range of *A. hagenowii* is unknown. *Aprostocetus hagenowii* currently has a cosmopolitan distribution but, it may have originated from Sub-Saharan Africa alongside its preferred host *P. americana* (Bell and Adiyodi 1982).

The objective of this study was to provide a more thorough examination of the host range for *A. hagenowii*. Cockroach species from three families (Blattidae, Ectobiidae, and Corydidae) with diverse geographic origins, natural histories, and oothecal morphologies were selected for testing. An effort was made to include non-pest species as well as pest species, and previously recorded hosts were also reexamined.

Methods and Materials

Wasps

Aprostocetus hagenowii wasps were provided by Dr. Barry Pawson (PNE, Inc., Tipp City, OH; Retired) and have been reared at Auburn University since 2020. The colony was maintained in 100 x 15 mm polystyrene Petri dishes on the oothecae of *P. americana*. *Aprostocetus hagenowii* is an arrhenotokous species (unmated females produce only male offspring), and a post emergence mating period of 24 h was used to allow for adequate mating time to occur before individual female wasps were separated into Petri dishes and provided with one or more fresh oothecae (aged 1 to 3 wk old). The dishes were then placed into a growth chamber [25 ± 2°C, 30–40% RH, and a 16:8 (L:D) photoperiod] for incubation. Adult wasps were not provided with food or water because the nutrients required for parasitization are sequestered during larval development.

Cockroaches

The cockroach species used over the course of the study came from numerous sources including online retailers, private collectors, and laboratory colonies. Their scientific and common names, authority, family, and reported status as a host for *A. hagenowii* are provided in Table 3.1. All cockroaches were provided a diet of Purina Laboratory Diet 5001 rat chow blocks, Purina Dog Chow (Ralston Purina, St. Louis, MO), and water *ad libitum*. Raw carrot, romaine lettuce, and Wardley Goldfish Flake Food (Hartz Mountain Corp., Secaucus, NJ) were occasionally provided to supplement diet. The following species were reared in 7.6 L (2.0 gal) plastic buckets to make oothecal collection easier: *P. americana*, *Blatta lateralis* (Walker), and *Supella longipalpa*

(Fab.). *Perplaneta americana* and *B. lateralis* were provided with loosely rolled 0.64 cm (0.25 in) 23-gauge hardware cloth for harborage. The bottom of the bucket which housed *P. americana* was removed and replaced with a layer of hardware cloth. Oothecae that fell through the openings were collected in a second bucket underneath. The buckets holding colonies of *S. longipalpa* contained hanging strips of canvas for harborage. The strips were suspended from a wire running through the center of rubber flask stoppers bolted to the upper bucket wall. *Arenivaga bolliana* (Sassure), *Polyphaga aegyptiaca* (L.) and *Po. sausseri* (Dohrn) were reared in plastic shoe boxes that were 35.6 cm L x 22.9 cm W x 12.7 cm H (14.0 in L x 9.0 in W x 5.0 in H) half-filled with ground coconut fiber. The tops of both boxes were covered by a screen held in place with rubber bands. *Parcoblatta lata* (Brunner von Wattenwyl) and *Pa. fulvescens* (Sassure and Zehntner) were reared in similar boxes that measured 34.3 cm L x 20.3 cm W x 12.7 cm H (13.5 in L x 8.0 in W x 5.0 in H), but a solid plastic lid was used to maintain the higher humidity preferred by these species.

The following species were reared in 1.9 L (0.5 gal) glass jars containing approx. 2.5 cm (1 in) of ground coconut fiber: *N. rhombifolia, N. propinqua* (Shelford), and *Deropeltis paulinoi* Bolivar. The remaining species were housed in 3.8 L (1 gal) glass jars: *Bl. germanica, Periplaneta japonica* Karny, *P. australasiae* (Fab.), *P. fuliginosa* (Serville), *P. brunnea* Burmeister, *B. orientalis*, *E. floridana*, and *E. lixa* Rehn. The species reared in glass jars were provided with corrugated cardboard harborage (flat sheets or rolls). Jars housing species preferring higher humidity were misted with water twice per week and provided with additional substrate such as leaflitter and additional ground coconut fiber 2.5 to 6 cm (1 to 2 in) deep. A band of mineral oil applied to the inner, upper surface of each jar prevented escape. The jar openings were covered with a layer of cloth mesh and a paper towel both held in place with

rubber bands. The oothecae of *N. rhombifolia*, *Pa. lata*, and *Pa. fulvescens* were especially prone to desiccation upon removal from their respective colony containers. Desiccation was prevented by placing the oothecae on moistened Kimwipes® (Kimberly-Clark, Neenah, WI) while in Petri dishes. All cockroach colonies and oothecae collected prior to exposure to *A. hagenowii* were held in a room maintained at 26 ± 2°C, 40–50% RH, and a 16:8 (L:D) photoperiod.

No-Choice Assays

The timing of wasp emergence and oviposition of oothecae heavily influenced the availability of each for testing. The no-choice assays entailed pairing 1-10 female *A. hagenowii* (aged at least 24 h post emergence) with individual oothecae in separate Petri dishes. The oothecae were aged ≤ 14 d, with the exception of *A. bolliana* oothecae, which were obtained from a colony under the care of another lab and without monitoring of oviposition dates. Oothecae were not previously exposed to oothecal parasitoids. Oothecae free of defects (i.e., broken or missing keel, dimples, or other visible abnormalities, etc.) were selected for experiments. However, the oothecae collected from the *P. japonica, P. brunnea, E. floridana, E. lixa*, and *N. rhombifolia* colonies were often malformed and dimpled limiting their use in the no-choice experiments. In these cases, oothecae with the fewest defects were selected for exposure to *A. hagenowii*. The oothecae of *Bl. germanica* were provided both attached and detached from the mother cockroach to determine if the presence of the mother deterred *A. hagenowii* from interacting with the ootheca.

Each assay was observed for 30 min after provisioning of an ootheca for signs of host investigation and acceptance (e.g., antennal drumming, ovipositor tapping, and attempted oviposition). The Petri dishes were then transferred into the wasp growing chamber for

incubation. Dishes in the incubator were checked every other hour during the first day of exposure for wasps displaying oviposition behavior. Upon the death of the wasps, the oothecae in the dishes were monitored for the emergence of wasps and nymphs. Dishes that produced wasps were marked with the emergence date, and dishes that produced cockroach nymphs were discarded. Dishes that produced neither wasps nor nymphs after two months of incubation were dissected to look for signs (e.g., larvae, pupae, or unemerged adult wasps) that *A. hagenowii* oviposition may have occurred.

Aprostocetus hagenowii appears to search for and interact with hosts more readily if many wasps are present simultaneously (Personal observations). Species that initially garnered little interest from *A. hagenowii* or which failed to produce wasps after oviposition behaviors were observed were selected for additional testing in *A. hagenowii* colony cages. These additional no-choice tests were performed by exposing multiple oothecae of the same cockroach species to *A. hagenowii* by placing them in an open Petri dish inside a Plexiglas stinging cage that housed numerous (≥ 60) *A. hagenowii* wasps of mixed sex and age. The stinging cage was held in the wasp growth chamber throughout each assay. The oothecae were observed for at least 30 min for signs (described above) of oviposition interest from the wasps. Periodic monitoring of the wasps for oviposition behavior was carried out until all of the wasps in the cage had died. The Petri dishes were then closed and incubated within the wasp growth chamber. Oothecae that produced wasps, nymphs, or neither were recorded, discarded, or dissected as described above.

Ootheca Cuticle Characteristics and Ovipositor Length

The oothecae produced by 7 cockroach species representing host and non-host species from each of the 3 families were selected for cuticle measurements. The majority of the species selected (*Bl. germanica, Pa. fulvescens, B. lateralis,* and *E. floridana*) were also used in the no-choice host range experiment. However, the oothecae of *P. americana* and *Po. saussurei* (Dohrn) were also used. *Periplaneta americana* was selected because it is the host used for maintaining the *A. hagenowii* colony, and *Po. saussurei* was used in place of its congener *P. aegyptiaca* due to the latter's colony and oothecae being destroyed by an infestation of dermestid beetles. The cuticle thickness of each species' oothecae was measured with an advanced onsite sensor (AOS) Absolute Digimatic electric caliper (Mitutoyo America Corp., Aurora, IL). The caliper was calibrated with precision stainless steel ring shims (McMaster-Carr, Elmhurst, IL), with the smallest tested shim being 0.1 mm thick. The manufacturer lists a tolerance of ± 0.013 for the 0.1 mm shim. The caliper measurements of the 0.1 mm shim were conducted in the same manner used to measure cuticle thickness (described below) and returned a mean thickness of 0.083 mm (Standard Error of the Mean (SEM) ± 0.002, N = 4); thus, 0.016 mm was added to measurements of oothecal cuticle thickness to compensate.

Oothecae were cut in half along and through the keel with a 5 mm micro knife (Fine Science Tools, North Vancouver, B.C.). Fine-tipped forceps were used to remove the oothecal contents from each half and to position the cuticle piece in the caliper jaws. Once in place, the caliper jaws were gently closed and placed within a 3-prong clamp. A support was placed beneath the caliper's main scale to hold it level with the clamp. The clamp screws were fully tightened around the caliper jaws, then loosened until pressure was no longer applied. The

resulting measurement was recorded and the process repeated two additional times with the same cuticle piece to obtain an average thickness. The areas below the keel, at the side, and bottom (Fig. 3.1) of each ootheca were measured to determine if cuticle thickness varied by location.

An ocular micrometer mounted to a dissecting microscope was calibrated with a slide micrometer and used to measure *A. hagenowii* ovipositor length. The ovipositor of *A. hagenowii* is held in a groove that runs the length of the abdomen and is shaped similarly to an insect or sewing pin. To isolate the ovipositor, the abdomen of a dead female *A. hagenowii* wasp was gently crushed, and the ovipositor was held in place with fine-tipped forceps while the abdominal debris were swept away with a paint brush. The length of each ovipositor was determined by measuring from the pointed tip of the distal end to just under the bulb (swelling) at the proximal end.

Statistical Analysis

Cuticle thickness measurements were analyzed with a Kruskal-Wallis ANOVA and Dunn's post hoc test in JASP (Version 0.16.3; JASP Team 2022) (Goss-Sampson 2020). Parasitism success was calculated as the proportion of exposed oothecae that produced wasps. The ANOVA and post hoc test results were compared to a threshold of $\alpha = 0.05$ and, with the exception of parasitism success, data are described as means ± SEM.

Results

Host Range

Aprostocetus hagenowii successfully parasitized three newly recorded host species: *B. lateralis*, *N. propinqua*, and *P. fulvescens*. Out of the 8 *B. lateralis* oothecae provided, 62.5% were successfully parasitized, and the average development time (time to emergence) for wasps within *B. lateralis* oothecae was 32.4 d. *Aprostocetus hagenowii* had a 16.0% parasitism success rate on *N. propinqua* oothecae and an average development period of 38.3 d. Lastly, parasitism success when provided *P. fulvescens* oothecae was 33.33%, and development time on this host species averaged 42.5 d. Among the previously recorded hosts that were reexamined, only *N. rhombifolia*, *P. australasiae*, and *B. orientalis* were successfully parasitized. Successful parasitization of *N. rhombifolia* (33.33%) only occurred when the oothecae were provided to *A. hagenowii* in the stinging cage. Due to a mis-match in wasp and oothecae availability, the number of *P. australasiae* oothecae available for testing was very limited, but the majority (66.67%) produced wasps. *Aprostocetus hagenowii* was most successful at parasitizing *B. orientalis* (77.78%). *Periplaneta brunnea*, *P. fuliginosa*, *P. japonica*, and *E. floridana* produced few oothecae that were free of defects (e.g., damaged keels, large dimples, etc.) limiting the number of replicates that could be provided to *A. hagenowii*. *Eurycotis lixa* and *Po. aegyptiaca*, had low replicate numbers due to low oothecal production, and *Bl. germanica* underwent limited testing due to challenges in keeping oothecae from desiccating after detachment from their mothers. Dissections of exposed oothecae that failed to produce wasps or cockroach nymphs after 2 months of incubation showed no signs of attempted parasitism.

Aprostocetus hagenowii displayed signs of interest (antennal drumming, ovipositor tapping) towards 15 of the 17 species of cockroach provided. Furthermore, *A. hagenowii*

displayed interest in oothecae despite the presence of defects. The oothecae of *S. longipalpa* and *Bl. germanica* (2 attached to mother and 3 detached) were exceptions as *A. hagenowii* would only make brief contact with their oothecae or ignore them completely. The replicate number of oothecae provided from each cockroach species is provided in Table 3.1.

Oothecal Cuticle Thickness and Ovipositor Length

The oothecae from the 7 species investigated had significantly different cuticle thicknesses for each zone measured [Kruskal-Wallis ANOVA: Below Keel ($H(5) = 21.762$, $P < 0.001$), Side ($H(6) = 34.111$, $P < 0.001$), Bottom ($H(6) = 34.299$, $P < 0.001$)] (Fig. 3.1). Two distinct groupings in thickness were apparent. *Polyphaga saussurei*, *E. floridana*, and *P. americana* had the thickest cuticles with the mean measurements for each zone > 0.08 mm (Table 3.2). *Neostylopyga propinqua*, *B. lateralis*, *Pa. fulvescens*, and *Bl. germanica* oothecae formed the second group with their mean cuticle thicknesses for each zone < 0.07 mm (Table 3.2). The zone below the keel of *B. germanica* oothecae was excluded from the results because it was too narrowly separated from the side of the ootheca for measurement with the calipers. The mean *A. hagenowii* ovipositor length was 0.92 ± 0.012 mm (N = 6).

Discussion

Seventeen species of cockroach were exposed to *A. hagenowii*. Three of these species (*B. lateralis*, *N. propinqua*, and *Pa. fulvescens*) are new host records, and three previously recorded host species (*B. orientalis*, *N. rhombifolia*, and *P. australiasiae*) are reconfirmed as hosts. *Blatta lateralis* and *N. propinqua* are unsurprising additions to the host range for *A. hagenowii*. Both are

members of Blattidae and have congeners (*B. orientalis* and *N. rhombifolia*) that were previously recorded as hosts (Pemberton 1941, Usman 1949). *Parcoblatta fulvescens* is an unusual host for *A. hagenowii*. It is a member of the Ectobiidae, endemic to North America (Atkinson et al. 1991), and rarely deposits its oothecae in locations accessible to *A. hagenowii* (Horn and Hanula 2002, Personal observations). *Aprostocetus hagenowii* appears able to locate oothecae buried under lightweight particles, such as coconut fiber, sand, or expanded polystyrene foam, but is unable to move those materials (Smith et al. 2022, Personal observations). *Parcoblatta fulvescens* as well as other *Parcoblatta* sp. can oviposit in moist substrates (e.g., soil, rotting logs, etc.) (Horn and Hanula 2002) that are likely to act as barriers to *A. hagenowii*. A non-native biological control organism parasitizing a native non-target species is concerning, but it is unlikely that *A. hagenowii* will have a meaningful impact on *Pa. fulvescens* populations owing to the latter's ootheca concealing behavior and the former's low parasitism rate when provided unconcealed *Pa. fulvescens* oothecae.

The initial parasitism success of *A. hagenowii* on *B. lateralis* oothecae led to the creation of an additional study (Smith et al. 2023) investigating the potential of *A. hagenowii* as a biological control for this cockroach, which is a pest of increasing concern throughout the US Southwest (Gaire and Romero 2020). Smith et al. (2023) were able to rear *A. hagenowii* for multiple generations using only *B. lateralis* oothecae for hosts. Similar attempts during the current study to rear *A. hagenowii* on *N. propinqua* oothecae for multiple generations failed to produce wasps beyond a second generation. Key differences between the oothecae of the two species are that *N. propinqua* oothecae are about half the size of *B. lateralis* oothecae and more prone to desiccation (personal observations), which may limit the viability of *N. propinqua* oothecae for *A. hagenowii* larval development.

There are many possible explanations for why *A. hagenowii* failed to parasitize 11 of the 17 species oothecae provided. Oothecal quantity and quality varied widely among the species tested. Low replicate number (≤ 5 oothecae) is one possible explanation for why parasitism was not observed in *P. brunnea*, *P. fuliginosa*, *P. japonica*, and *E. floridana* despite their being previously recorded hosts. The *A. hagenowii* strain used in this study has been reared on *P. americana* oothecae for over 30 years, which may have affected its ability to switch to other species of host (Smith et al. 2023). The characteristics of the oothecal cuticle, the environment within the ootheca, and the composition of each species' eggs likely also play roles in host acceptance and parasitism success. Cuticle thickness alone is unlikely to be a barrier to parasitism for *A. hagenowii* as the mean ovipositor length (0.92 ± 0.012 mm) was far longer than the thickest cuticles measured. The hardness or flexibility of the cuticle may prevent the ovipositor from puncturing an ootheca, but these aspects of the cuticle have yet to be studied. The oothecal cuticles of the Corydiidae species used in this study felt particularly thick and sturdy compared to the cuticles of Ectobiidae cockroaches, which were thin and easily broken when handled. The cuticles of Blattidae oothecae ranged in thickness and flexibility between the two other families (Figs 3.2-3.4).

Several molecules (e.g., calcium oxalate and protocatechuic acid) associated with the oothecae and exuviae of *P. americana* act as kairomones for *A. hagenowii* host location and acceptance (Suiter et al. 1996). Differences among cockroach species in the presence and quantity of these molecules is not well studied, but Kramer et al. (1991) found significantly higher proportions of calcium oxalate in the oothecae of the known hosts *P. americana*, *P. fuliginosa*, and *B. orientalis* (7-8%) compared to the non-host *Bl. germanica* (< 1%).

The environment within the ootheca, especially moisture content, may play a more important role than the oothecal cuticle for *A. hagenowii* success. The host species that *A. hagenowii* performed best against (*P. americana* and *B. orientalis*) require little to no absorption of moisture from the surrounding environment to complete development (Roth and Willis 1955). Conversely, oothecae collected from *Pa. lata, Pa. fulvescens,* and *N. rhombifolia* quickly shriveled upon removal from moist substrate, and the oothecae of *B. germanica* also desiccated rapidly once removed from the mother. The oothecae of *Po. aegyptiaca, Po. saussueri,* and *A. bolliana* contained almost no fluid surrounding the eggs. Roth (1967) noted that the oothecae of *Poly. aegypticaca* and three species of *Arenivaga* had about half as much water content (32-37%) as the oothecae of *B. orientalis*, *N. rhombifolia*, and numerous *Periplaneta spp*. (59-67%). However, water content alone does not appear to be a deciding factor as the oothecae of *S. longipalpa* (a non-host) maintain a high fluid content throughout their development (Roth 1967).

Information on the differences in the composition of cockroach eggs is severely lacking. Studies of *Blattabacterium* spp. cockroach symbionts have found most, if not all, species of cockroach carry a unique strain of the bacterium (Patiño-Navarrete 2013, Noda et al. 2020). How *Blattabacterium* spp. or other symbionts may affect developing *A. hagenowii* larvae is unknown. Likewise, the influence that egg nutritional composition may have on *A. hagenowii* development is also unknown. Ultimately, determining what factors prevent *A. hagenowii* from utilizing one host species versus another will require gaining a better understanding of the cockroach ootheca beyond the physical barrier of the cuticle.

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References

Atkinson TH, Koehler PG, Patterson RS. Catalog and atlas of the cockroaches (Dictyoptera) of North America North of Mexico. Misc. Publ. Entomol. Soc. Am. 1991:78:1–86.

Bell WJ, Adiyodi KG. The American cockroach. London (UK): Chapman and Hall; 1982.

Bell WJ, Roth LM, Nalepa CA. Cockroaches: ecology, behavior, and natural history. Baltimore (MD): The Johns Hopkins University Press; 2007.

Dai XH. Studies on the stimulating behaviour of rectal secretions of cockroach on the oviposition behaviour of *Tetrastichus hagenowii* (Hymenoptera, Eulophidae). Chin. J. Biol. Control 1992:8(1):13-15

Edmunds LR, Some notes on the habits and parasites of native wood-roaches in Ohio (Orthoptera: Blattidae). Ent. News. 1952:63(6):11.

Gaire S, Romero A. Comparative efficacy of residual insecticides against the Turkestan cockroach, *Blatta lateralis*, (Blattodea: Blattidae) on different substrates. Insects 2020:11(8):1-9.

Goss-Sampson MA. *Statistical analysis in JASP: a guide for students*. 5th ed. 2022. https://jasp-stats.org/wp-content/uploads/2022/04/Statistical-Analysis-in-JASP-A-Students-Guide-v16.pdf.

Hagenbuch BE, Patterson RS, Koehler PG, Brenner RJ. Mass production of the cockroach oothecal parasitoid, *Tetrastichus hagenowii* [Hymenoptera : Eulophidae], and its host, the American cockroach [Orthoptera : Blattidae]. J. Econ. Entomol. 1988:81(2):531-535.

Harlan HJ, Kramer RD. Limited host specificity in *Tetrastichus hagenowii* (Ratzburg). J. Ga. Entomol. Soc. 1980:16(1):64-67.

Horn S, Hanula JL. Life history and habitat associations of the broad wood cockroach, *Parcoblatta lata* (Blattaria: Blattellidae) and other native cockroaches in the coastal plain of South Carolina. Ann. Entomol. Soc. Am. 2002:95(6):665-671.

LeBeck L. A review of the hymenopterous natural enemies of cockroaches with emphasis on biological control. Entomophaga 1991:36(3):335-352.

Narasimham AU, Sankaran T. Domiciliary cockroaches and their oothecal parasites in India. Entomophaga 1979:24(3):273-279.

Noda T, Okude G, Meng XY, Koga R, Moriyama M, Fukatsu T. Bacteriocytes and *Blattabacterium* endosymbionts of the German cockroach *Blattella germanica*, the forest cockroach *Blattella nipponica*, and other cockroach species. J Zool. 2020:37(5):399–410.

Patiño-Navarrete R, Moya A, Latorre A, Peretó J. Comparative genomics of *Blattabacterium cuenoti*: the frozen legacy of an ancient endosymbiont genome. Genome Biol Evol. 2013:5(2):351–361.

Pawson BM, Gold RE. Evaluating different release strategies for the control of American cockroaches using the egg parasitoid, *Aprostocetus hagenowii*, In Wildey KB and

Robinson WH, editors. Proceedings of the First International Conference on Urban Pests; Cambridge, UK.; 1993 p. 407-413.

Pemberton CE. Notes and exhibitions. Proc. Haw. Entomol. Soc. 1941:11:19.

Roth LM. Water changes in cockroach oothecae in relation to the evolution of ovoviviparity and viviparity. Ann. Entomol. Soc. Am. 1967:60(5):928-946.

Roth LM, Willis ER. The biology of the cockroach egg parasite, *Tetrastichus hagenowii*. Trans. Amer. Entomol. Soc. 1954:80(2):53-72.

Roth LM, Willis ER. Water content of cockroach eggs during embryogenesis in relation to oviposition behavior. J. Exp. Biol. 1955:128(3):489-509.

Roth LM, Willis ER. The biotic associations of cockroaches. Smithson. Misc. Publ. 1960:141:1-470.

Slade EM, Ong XR. The future of tropical insect diversity: strategies to fill data and knowledge gaps. Curr. Opin. Insect. Sci. 2023: Journal Pre-Proof: 23pgs.

Smith CM, Chicas-Mosier AM, Appel AG, Fadamiro HY. Gravid *Periplaneta americana* (Blattodea: Blattidae) fails to detect or respond to the presence of the oothecal parasitoid *Aprostocetus hagenowii* (Hymenoptera: Eulophidae). Environ Entomol. 2022:51(6):1086–1093.

Smith CM, Chicas-Mosier AM, Fadamiro HY, Appel AG. Potential of the oothecal parasitoid *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) as a biological control agent for the Turkestan cockroach (Blattodea: Blattidae). J. Econ. Entomol. 2023:In advance publication: https://doi.org/10.1093/jee/toad078.

Suiter DR, Carlson DA, Patterson RS, Koehler PG. Host-location kairomone from *Periplaneta americana* (L.) for parasitoid *Aprostocetus hagenowii* (Ratzeburg). J. Chem. Ecol. 1996:22(4):637-651.

Tee HS, Saad AR, Lee CY. Evaluation of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) for the control of American cockroaches (Dictyoptera: Blattidae) in sewers and crevices around buildings. J. Econ. Entomol. 2011:104(6):2031-2038.

Usman S. Some observations on the biology of *Tetrastichus hagenowii* Ratz. an egg parasite of the house-cockroach (*Periplaneta americana* L.). Curr. Sci. 1949:18(11):407-408.

Van Driesche RG. Assessing host ranges for parasitoids and predators used for classical biological control: a guide to best practices. In: Van Driesche RG, Murray T, Reardon R, editors. Chapter 1. Introduction: Predicting host ranges of parasitoids and predacious insects – what are the issues? Morgantown (WV): USDA Forest Health Technology Enterprise Team; 2004. p. 1-3.

Tables and Figures

Table 3.1. A list of the cockroach species, number of oothecae from each exposed to *A. hagenowii*, host status, and parasitism success during host range testing.

Species and Authority	n	Common Name	Family	Documented as host	Parasitism Success %
Blatta lateralis (Walker)	8	Turkestan Cockroach	Blattidae	N	62.5%
Blatta orientalis L.	9	Oriental Cockroach	Blattidae	P, R	77.8%
Deropeltis paulinoi Bolívar	6	Ornate Velvet Cockroach*	Blattidae	-	
Neostylopyga propinqua (Shelford)	25	African Bullet Cockroach*	Blattidae	N	16.0%
Neostylopyga rhombifolia (Stoll)	6	Harlequin Cockroach	Blattidae	P, R	33.3%
Periplaneta australasiae (Fab.)	3	Australian Cockroach	Blattidae	P, R	66.7%
Periplaneta brunnea Burmeister	3	Brown Cockroach	Blattidae	P	
Periplaneta fuliginosa (Serville)	3	Smokybrown Cockroach	Blattidae	P	
Periplaneta japonica Karny	3	Japanese Cockroach*	Blattidae	P	
Eurycotis floridana (Walker)	4	Florida Woods Cockroach*	Blattidae	P	
Eurycotis lixa Rehn	4	Hustler Cockroach*	Blattidae	-	
Arenivaga bolliana (Saussure)	7	Boll's sand cockroach*	Corydiidae	-	
Polyphaga aegyptiaca (L.)	5	Egyptian Desert Cockroach*	Corydiidae	-	
Blattella germanica (L.)	5	German Cockroach	Ectobiidae	-	
Parcoblatta fulvescens (Saussure and Zehntner)	6	Fulvous Wood Cockroach*	Ectobiidae	N	33.3%
Parcoblatta lata (Brunner von Wattenwyl)	16	Broad Wood Cockroach*	Ectobiidae	-	
Supella longipalpa (Fab.)	17	Brownbanded Cockroach	Ectobiidae	-	

Species with common names marked by an asterisk have not been assigned official common names by the Entomological Society of America as of June 2023. P = Previously recorded as host, R = Reconfirmed as host, - = not previously recorded as host, and N = newly recorded host.

Table 3.2. Mean oothecal cuticle thickness measurements of several cockroach species.

Species (n)	Below Keel (mm ±SEM)	Side (mm ±SEM)	Bottom (mm ±SEM)
Blatta lateralis (6)	0.065 ± 0.005	0.057 ± 0.004	0.052 ± 0.004
Blattella germanica (6)	N/A	0.027 ± 0.001	0.027 ± 0.002
Eurycotis floridana (4)	0.093 ± 0.007	0.110 ± 0.007	0.110 ± 0.006
Neostylopyga rhombifolia (5)	0.059 ±0.007	0.050 ± 0.004	0.058 ± 0.003
Parcoblatta fulvescens (6)	0.047 ± 0.011	0.043 ± 0.005	0.050 ± 0.008
Periplaneta americana (7)	0.105 ± 0.007	0.087 ± 0.010	0.081 ± 0.008
Polyphaga sausserei (8)	0.098 ± 0.008	0.112 ± 0.004	0.120 ± 0.005

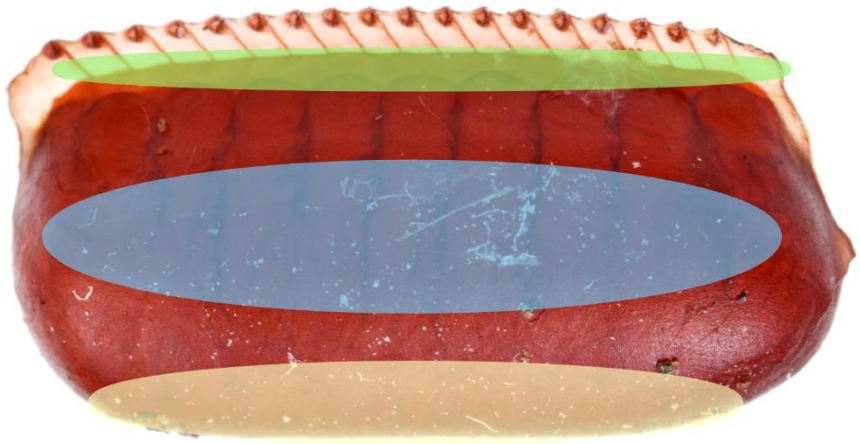

Fig. 3.1. Diagram of areas measured to determine thickness of the oothecal cuticle: Green – Below Keel, Blue – Side, and Orange – Bottom. Figure adapted from image by Salvador Vitanza and Texas A & M AgriLife Extension.

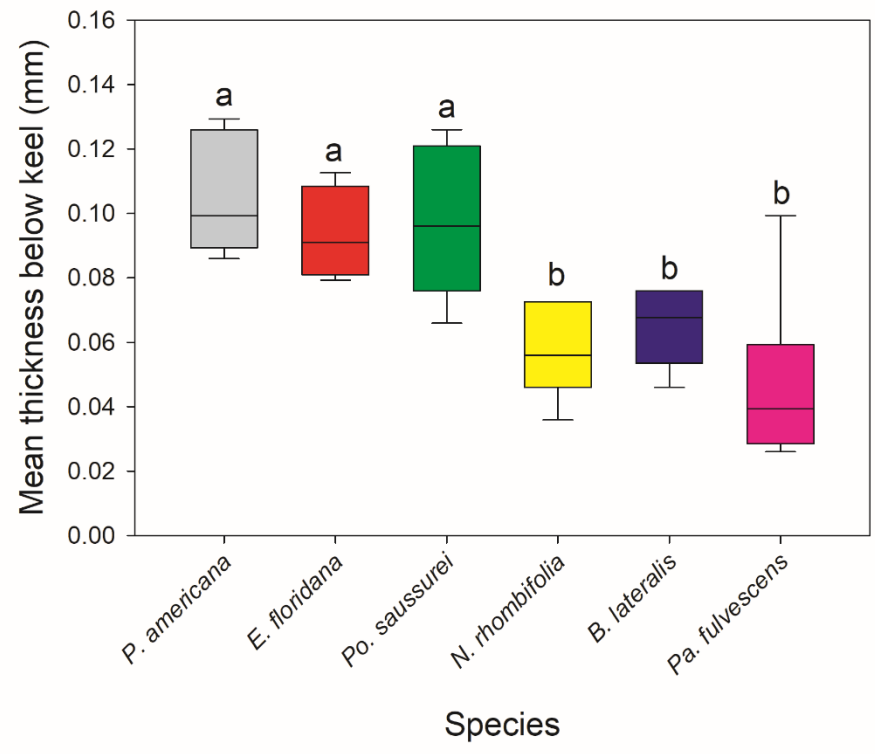

Fig. 3.2. Kruskal-Wallis ANOVA and Dunn's post hoc comparison of mean oothecal cuticle thickness measurements below the keel. The below keel zone of *Bl. germanica* is excluded as it was too narrow to measure. Boxes that do not share letters are significantly different with $P \leq 0.05$. Replicate number: *P. americana* = 6, *E. floridana* = 4, *Po. saussurei* = 8, *N. rhombifolia* = 5, *B. lateralis* = 6, and *Pa. fulvescens* = 6.

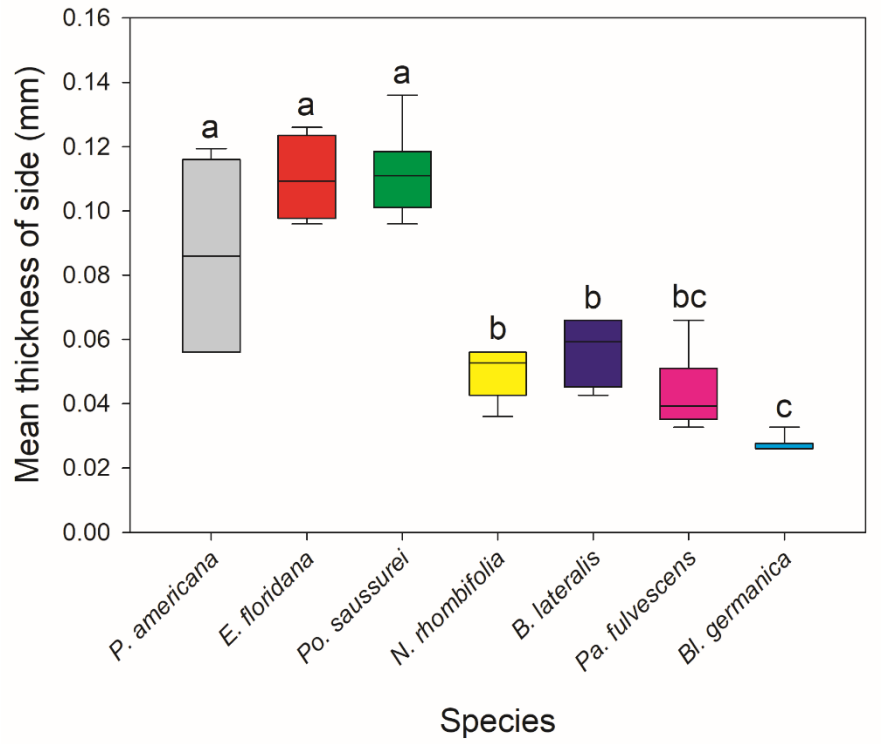

Fig 3.3. Kruskal-Wallis ANOVA and Dunn's post hoc comparison of mean oothecal cuticle side thickness. Boxes that do not share letters are significantly different with $P \leq 0.05$. Replicate number: *P. americana* = 6, *E. floridana* = 4, *Po. saussurei* = 8, *N. rhombifolia* = 5, *B. lateralis* = 6, *Pa. fulvescens* = 6, and *Bl. germanica* = 6.

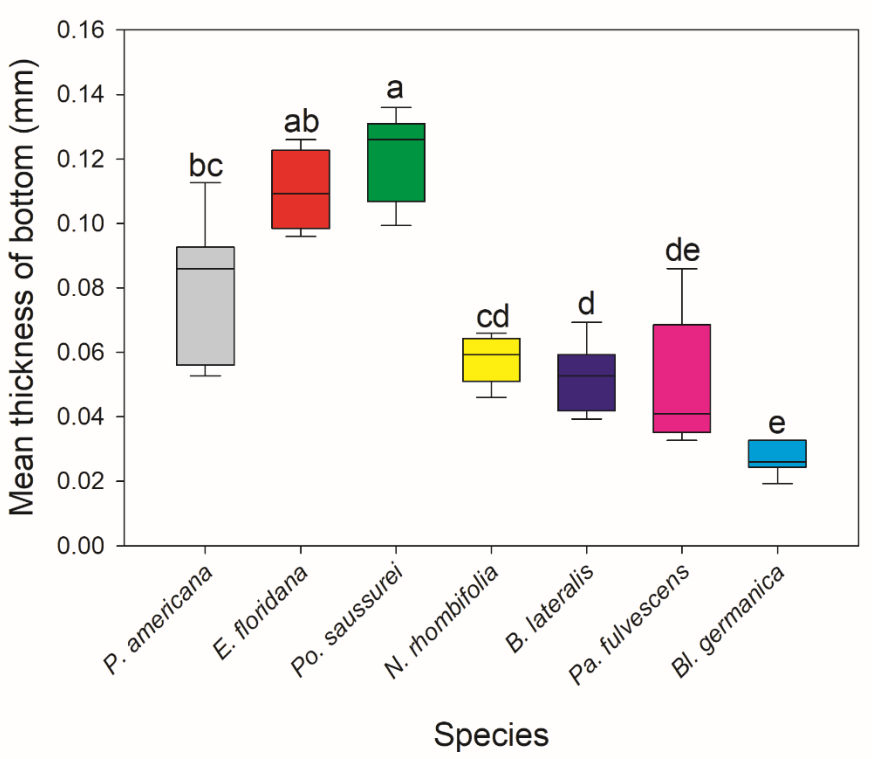

Fig 3.4. Kruskal-Wallis ANOVA and Dunn's post hoc comparison of mean oothecal cuticle bottom thickness. Boxes that do not share letters are significantly different with $P \leq 0.05$. Replicate number: *P. americana* = 6, *E. floridana* = 4, *Po. saussurei* = 8, *N. rhombifolia* = 5, *B. lateralis* = 6, *Pa. fulvescens* = 6, and *Bl. germanica* = 6.

Chapter 4: Potential of the oothecal parasitoid *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) as a biological control agent for the Turkestan cockroach, *Blatta lateralis* (Blattodea: Blattidae)

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Abstract

The Turkestan cockroach, *Blatta lateralis* (Walker), is a peridomestic pest of growing concern in the US Southwest. The parasitoid *Aprostocetus hagenowii* (Ratzburg) is used in IPM programs targeting other blattid cockroach species and may aid in *B. lateralis* suppression. Information about the ability of *A. hagenowii* to parasitize *B. lateralis* is lacking. A no-choice host-switching experiment was used to test *A. hagenowii* acceptance of *B. lateralis* oothecae, and a multi-generational no-choice experiment was used to determine the suitability of *B. lateralis* as a host for *A. hagenowii* over several months of rearing. *Periplaneta americana* (L.) (Blattodea: Blattidae), the preferred host of *A. hagenowii*, and *Blatta orientalis* L., a known host and relative of *B. lateralis*, were used for comparison. Development time was similar among hosts and generations ($P > 0.05$). Parasitism success and proportion of female progeny declined significantly with subsequent generations on both *Blatta* spp. (parasitism success: $\chi^2 = 14.916$; df = 2; $P = 0.001$; proportion female: $H = 6.364$; df = 2; $P = 0.041$). These results suggest that *A. hagenowii* may initially aid in suppression of *B. lateralis*, but an overall decline in fitness will require repeated releases or provisioning of *P. americana* oothecae. Development of a strain more suitable for *B. lateralis* control may be possible via selection from laboratory strains or through use of wild *A. hagenowii* from areas where *B. lateralis* is present.

Introduction

The Turkestan cockroach, *Blatta lateralis* (Walker), is a peridomestic pest with a native range extending from North Africa to Central Asia (Kim and Rust 2013). Aided by global trade and military movements in the Middle East, *B. lateralis* has been introduced to numerous regions including the United States (USDA 1978, Olson 1985, Kim and Rust 2013), Mexico (Cueto-Medina et al. 2015), Spain (Miralles-Núñez et al. 2020), Japan (Sumino et al. 2006), Cyprus, and Sardinia (Davranoglou et al. 2020). *Blatta lateralis* has become well established in the southwestern United States where it is common near humid microhabitats (i.e., drains, water meter boxes, leaf litter, potted plants, and dumpsters) (Kim and Rust 2013, Gaire and Romero 2020). Anecdotal observations made by pest management professionals in California have noticed a sharp increase in calls seeking *B. lateralis* control in homes and warehouses (Harbison 2022). Notably, *B. lateralis* appears to be displacing its relative the Oriental cockroach, *B. orientalis* L., and the American cockroach, *Periplaneta americana* (L.) (Blattodea: Blattidae), in areas of the Southwestern US where they had long been the dominant peridomestic cockroach pests (Hebard 1917, Kim and Rust 2013, Harbison 2022). The taxonomic and phylogenetic relationship between *B. lateralis* and its relatives in the Blattinae is uncertain (Deng et al 2023) and remains unsettled. Therefore, we will continue to refer to this species as *B. lateralis* as it is accepted by the Entomological Society of America.

The presence of cockroaches is associated with lower home values, heightened psychological stress, and social stigmatization for a building's residents (Shah et al. 2018, Gondhalekar et al. 2021). Cockroaches in homes, hospitals, and food processing facilities pose a health risk as they can harbor and transmit parasites and pathogens (e. g., Acanthocephala, hepatitis B, *Salmonella* spp., *Staphylococcus* spp., etc.) and are attracted to food and food

preparation areas (Roth and Willis 1960, Donkor 2019, Nasirian 2019). Furthermore, cockroaches are associated with several allergy related conditions, such as asthma and eczema, which can be triggered by exposure to feces and exuviae (Pomés et al. 2019, Huang et al. 2021).

Effective control of peridomestic cockroaches requires integrated pest management (IPM) techniques aimed at reducing outdoor populations and preventing their movement into buildings (Hagenbuch et al. 1988, Smith et al. 1995, Hedges 2021). Such management typically involves removing outdoor harborage sites along with application of granular baits and insecticide barrier sprays applied to building perimeters (Smith et al. 1995, Tee et al. 2011, Hedges 2021). Barrier sprays can be highly effective deterrents but quickly lose their potency in hot climates, such as those found in the southern regions of the US, requiring regular reapplication (Smith et al. 1995). Bait and barrier treatments on the exterior of a building may also fail to address cockroach incursions that originate from crawl spaces, interior drains, pipe chases, connections between buildings, tree holes, and other outdoor habitats that allow cockroaches to bypass treated areas (Pawson and Gold 1993, Hedges 2021). While insecticides are effective at killing cockroach nymphs and adults in and around homes, the cuticle of the ootheca (egg case) protects the eggs within from chemical applications (Bell et al. 1998, Bressan-Nascimento et al. 2008, Tee et al. 2011).

Inclusion of natural enemies, such as parasitoid wasps, that target cockroach oothecae can improve IPM program success (LeBeck 1991, Narasimham 1992, Tee et al. 2011). *Aprostocetus hagenowii* (Ratzburg) (Hymenoptera: Eulophidae) is an oothecal parasitoid that attacks numerous cockroach species, primarily in the family Blattidae (LeBeck 1991). *Periplaneta americana* is its preferred host, but it can also utilize oothecae of other *Periplaneta* spp. as well as *B. orientalis, Eurycotis floridana* (Walker), and *Neostylopyga rhombifolia* (Stoll)

(Narasimham 1984, LeBeck 1991, Pawson and Gold 1993). This species is simple to rear under laboratory conditions, and its gregarious nature and high fecundity are conducive for mass rearing (Narasimham 1984, Smith et al. 2022). Introduction of *A. hagenowii* can support immediate and long-term cockroach suppression as they actively seek out and destroy oothecae concealed in hard to access areas (e. g., wall voids and pipe chases) or within materials such as expanded polystyrene (Styrofoam) (Pawson and Gold 1993, Smith et al. 2022). Furthermore, the presence of *A. hagenowii* appears to go unnoticed by cockroaches, and their use should not impact the responses of cockroaches to other treatment methods (e.g., baits, repellants, traps, etc.) (Smith et al. 2022). *Aprostocetus hagenowii* may aid in *B. lateralis* management, but there has yet to be an investigation of whether this parasitoid can successfully utilize *B. lateralis* as a host.

The goals of this study were three-fold. First, to determine if *A. hagenowii* would accept *B. lateralis* oothecae as hosts. Second, to compare *B. lateralis* parasitization success with that of known hosts. Last, to determine if *A. hagenowii* could be reared for multiple generations on the oothecae of *B. lateralis*. Considering that *A. hagenowii* attacks many blattid species, including *B. orientalis*, we hypothesized *B. lateralis* oothecae would be accepted as a host and at a similar rate of parasitization as *P. americana* and *B. orientalis*. Furthermore, Pawson and Gold (1993) indicate that *A. hagenowii* can be reared for many generations exclusively on the oothecae of *B. orientalis*. Similar results were expected with *B. lateralis*.

Materials and Methods

Insect Rearing

The *B. lateralis* used in this study have been reared at Auburn University since 1990 and have never been exposed to insecticides. During spring 2020, adult females were collected from the primary colony and housed in a 7.6 L (2.0 gal) plastic bucket for easier collection of oothecae. The lid of the bucket contained a 16.2 cm (6.0 in) diameter opening covered by aluminum screen and a layer of paper towel. The screen was held in place with hot glue, and the paper towel was held by masking tape. Mineral oil was applied to the upper 5 cm of the inner wall of the bucket. The arrangement of the screen, paper towel, and mineral oil allowed for ventilation in the bucket while preventing the escape of the cockroaches. A loosely rolled tube of 0.64 cm (0.25 in) 23-gauge metal screening was provided as harborage. Similar to *B. lateralis*, adult female *P. americana* were collected from the primary colonies in spring 2020 and reared in a bucket with hardware cloth for harborage. The *P. americana* used in this study came from colonies that have been reared at Auburn University since 1985 and have never been exposed to insecticides. The *B. orientalis* used for this study had been reared at Auburn University since 2020. They were housed in a 3.8 L (1 gal) glass jar, with a rubber band to secure fine mesh fabric and a layer of paper towel over the jar's mouth. The upper 5 cm of the jar interior was thinly coated with petroleum jelly as the primary means of preventing escape. Rolled and stapled tubes of corrugated cardboard were provided as harborage.

All three cockroach species were provided a diet of Purina Laboratory Diet 5001 rat chow blocks, Purina Dog Chow Complete Adult chicken flavor formula (Ralston Purina, St. Louis, MO), and water *ad libitum*. Colonies were kept in a rearing room maintained at 27 ± 2°C, 45–50% RH, and a 16:8 (L:D) photoperiod. Each week all oothecae present in each colony were

collected. Oothecae showing signs of damage (i.e., broken keel, large dimples, etc.) were discarded, while those in acceptable condition were separated by species and held in 100 x 15 mm polystyrene Petri dishes (Thermo Fisher Scientific, Waltham, MA). The lid of each dish was marked with the species and date of collection of each ootheca and stored in a growth chamber at 25 ± 2°C, 30–40% RH, and a 16:8 (L:D) photoperiod until needed.

Aprostocetus hagenowii wasps were obtained from Dr. Barry Pawson (PNE, Inc., Tipp City, OH; Retired), have been maintained at Auburn University since 2020, and were reared according to Smith et al. (2022). The colony was held in 100 x 15 mm polystyrene Petri dishes on the oothecae of *P. americana*. Parasitoids were incubated within a growth chamber at 25 ± 2°C, 30–40% RH, and a 16:8 (L:D) photoperiod. *Aprostocetus hagenowii* produce only male offspring if unmated (arrhenotokous), thus, a post emergence mating period of 24 h was used before individual female wasps were each separated into Petri dishes and provided with an ootheca. *Aprostocetus hagenowii* will accept oothecae from a wide age range but prefer older, rather than newly laid, hosts (Narasimham 1984). Thus, only oothecae aged between 1 to 3 wk old were provided. Adult wasps were not provided with food or water as they obtained the nutrients necessary for reproduction during larval development. Provisioning of food will extend the lifespan of adult *A. hagenowii*, but the urban environments they are often released into, such as drains, pipe chases, steam tunnels, and sewers, may lack food resources (Narasimham 1984, Pawson and Gold 1993, Tee et al. 2011).

Host Switching

The host switching no-choice experiment consisted of pairing single female *A. hagenowii* (Parent Generation), all reared on the oothecae of *P. americana*, with individual oothecae from *P. americana* (control), *B. orientalis* (known host), and *B. lateralis*. After each wasp was paired

with an ootheca in a Petri dish (forming an experimental unit), the dish was placed in a growth chamber at 25 ± 2°C, 30–40% RH, and a 16:8 (L:D) photoperiod. Dishes were monitored for the emergence of parasitoids and nymphs. Oothecae that failed to hatch within 2 months of the exposure date were dissected and examined for evidence (i.e., larvae, pupae, or adult wasps) that parasitization had occurred.

Multi-generation Rearing

Wasps that emerged from the oothecae parasitized in the no-choice experiment above were regarded as Generation 1. After a 24 h mating period, individual females from Generation 1 were isolated and each provided a single ootheca to continue their respective host lines. The wasps that emerged from this next phase were regarded as Generation 2. This process was repeated to obtain Generation 3. A similar procedure was followed using wasps reared on *P. americana*. However, because *A. hagenowii* has been reared on *P. americana* for many years, there is an unknown number of generations on this host. For this reason, progeny reared on *P. americana* were not separated into generations during the experiment. Multi-generation rearing on each host species was conducted simultaneously.

The wasps produced over the course of the experiment were held in their respective Petri dishes, each dish acting as an experimental unit, until their death. To determine the effect of host and generation on wasp body size, the mean hind tibia and forewing lengths were calculated for the wasps in each experimental unit. Measurement of leg and wing lengths were selected over body length as they are less likely to deform after death. Thirty randomly selected individuals of each sex from every experimental unit were selected for measurements. If an experimental unit produced fewer than 30 individuals of a particular sex, all individuals were measured. A Mantis

stereo microscope (Vision Engineering Ltd., Send, UK) and uEye Cockpit software (Imaging Development Systems GmbH, Obersulm, Germany) were used to make measurements at 10x magnification. Before measuring, the uEye Cockpit's digital ruler was calibrated with a physical ruler. The same physical ruler was used for each calibration. Data regarding parasitism success, development time, progeny number, and proportion of female wasps were also recorded.

Statistical Analysis

The generalized linear mixed model in SAS (Proc Glimmix, SAS 9.4, SAS Institute Inc., Cary, NC) was used to examine the effects of host species and generation (alone and in combination) on forewing and hind tibia lengths. A gamma distribution and least-squares means were used to compensate for deviation from normality and uneven number of replicates, respectively. For comparison with Generations 2 and 3 of *B. orientalis* and *B. lateralis*, multiple *P. americana* replicates (each containing 30 individual of each sex) were constructed using a random number generator to select wasps from Generation 1. Randomly selected wasps were not used more than once within each constructed generation. Data for proportion female progeny failed checks for normality and were analyzed with a Kruskal-Wallis ANOVA and Dunn post-hoc test in JASP version 0.16.3 (JASP Team, Amsterdam, The Netherlands). Parasitization success was analyzed using odds ratio analysis in SAS followed by a chi-square post-hoc analysis. The N-1 chi-square test was used as an adjustment for the smaller sample sizes in generations 2 and 3 (Campbell 2007). Development time (days) data were not normally distributed and were analyzed with a Kruskal-Wallis ANOVA and Dunn post-hoc test in JASP. Parasitization success and development time analyses were restricted to within species comparisons beyond Generation 1

due to *P. americana* having one generation. Statistical analysis was compared to a threshold of α = 0.05 for all analyses. Data are described as means ± SEM (standard error of the mean).

Results

Host Switching

Generation 1

Aprostocetus hagenowii wasps that were switched from *P. americana* to *B. lateralis* or *B. orientalis* successfully parasitized the majority of provided oothecae (66.7%, N = 27 and 93.3%, N = 15, respectively) (Fig. 4.1). While success on *B. orientalis* oothecae was notably greater compared to *B. lateralis*, there was no significant difference in success among these host species (Odds Ratio = 0.143; 95% CI = 0.016 – 1.265; P = 0.080). There was also no significant difference in success among either *Blatta* species or the *P. americana* control (61.5%, N = 26) [χ^2 = 4.963; df = 2, 68; P = 0.084)]. Among the oothecae that were not successfully parasitized, most produced nymphs (23.5%) and a small proportion failed to produce either wasps or nymphs (5.9%). Dissection of the oothecae that failed to hatch showed no evidence of attempted parasitization.

There was no significant difference in development time (days) of *A. hagenowii* among host species: *B. lateralis* = 35.94 ± 1.95 d, *B. orientalis* = 39.25 ± 5.16 d, and *P. americana* = 33.71 ± 1.09 d [ANOVA F = 169.363; df = 2; P = 0.324]. The average number of progeny produced from each host was not significantly different: *B. lateralis* = 53.63 ± 5.14, *B. orientalis* = 62.92 ± 7.47, and *P. americana* = 48.40 ± 5.59 [ANOVA F = 1421.50; df = 2; P = 0.275]. There was also no significant difference in the proportion of female progeny: *B. lateralis* = 63.69

± 7.95%, *B. orientalis* = 61.35 ± 10.55, and *P. americana* = 66.75 ± 9.80 [Kruskal-Wallis ANOVA H = 0.161; df = 2; P = 0.923].

Multi-generation Rearing

The parasitization success of *A. hagenowii* declined with each generation of rearing on both *Blatta* species (Fig. 4.1). While the parasitization success of Generation 1 wasps on *B. lateralis* was not significantly lower than the Parent Generation (P > 0.05), Generation 2 wasps were significantly less successful than the Parent Generation (χ^2 = 5.25; df = 2, 36; P = 0.02). The decline in parasitism success was significant with each successive generation of rearing on *B. orientalis* (Parent – Generation 1: χ^2 = 8.13; df = 2, 38; P = 0.004; Parent – Generation 2: χ^2 = 9.63; df = 2, 22; P = 0.002) (Fig. 4.1). Seven of the *B. lateralis* oothecae and 10 of the *B. orientalis* oothecae exposed to the wasps from Generation 1 produced nymphs. Among the 9 *B. lateralis* and 7 *B. orientalis* oothecae provided to Generation 2 wasps, 5 from each host species hatched out nymphs. Dissection of the oothecae that produced neither wasps nor cockroach nymphs contained no observed evidence of attempted parasitism.

Wasp development time was similar across generations for each species: *P. americana* = 33.71 ± 1.09 d, *B. lateralis* = 35.18 ± 1.55 d, and *B. orientalis* = 36.83 ± 2.34 d (P > 0.05). With generations combined, host species did not have a significant effect on the proportion of female progeny produced by *A. hagenowii*: *P. americana* = 67.14 ± 7.19%, *B. lateralis* = 58.80 ± 7.62%, and *B. orientalis* = 51.51 ± 8.27 % (P > 0.05). However, excluding *P. americana*, generation did have a significant effect on proportion of female progeny produced within each host line [Kruskal-Wallis ANOVA (H = 4.069; df = 1; P = 0.044) with Dunn's post hoc: Generation 1-2: z = 2.017; P = 0.022] (Fig. 4.2). While there were similar proportions of female

progeny produced for *B. orientalis* Generations 2 (41.66 ± 12.53%) and 3 (40.32 ± 40.32%) and *B. lateralis* Generation 2 (43.34 ± 19.38%), only 2.98 ± 2.98% of the wasps from *B. lateralis* Generation 3 were female (data not shown). The very low number of oothecae (two from each host species) successfully parasitized in Generation 3 limits their use in statistical analyses, and the large SEM for *B. orientalis* Generation 3 is an artifact due to this small sample size.

Wasp Size

The hind tibia and forewings of 2,453 wasps were measured. The mean (least squares) lengths of these body parts for both female and male *A. hagenowii* are provided as Table 4.1. Body part lengths were influenced by host, generation, and host x generation interaction (all $P < 0.01$). Hind tibia and forewings of female *A. hagenowii* reared on *B. orientalis* and *B. lateralis* had similar lengths ($p > 0.05$) but were significantly longer compared to females reared on *P. americana* ($P < 0.05$) (Table 4.2). In contrast, male *A. hagenowii* reared on *B. lateralis* were not significantly different from males reared on *P. americana* ($P > 0.05$) but were significantly smaller than those reared on *B. orientalis* ($P < 0.001$) (Table 4.2).

Comparisons of mean hind tibia and forewing lengths among hosts and generations are shown in Figs. 4.3 and 4.4. Female and male mean hind tibia and forewing lengths of *A. hagenowii* were similar across generations on *P. americana* ($P > 0.05$). The size of both body parts was also similar among generations for females reared on *B. orientalis* (all $P > 0.05$). Males reared on *B. orientalis* maintained similar forewing lengths across generations ($P > 0.05$), but mean hind tibia length declined significantly for Generation 2 ($t = 4.46$; $df = 848$; $P < 0.001$). Mean hind tibia and forewing lengths of female wasps reared on *B. lateralis* increased significantly from Generation 1 to 2 (hind tibia [$t = -5.48$; $df = 1376$; $P < 0.001$]; forewing [$t = -$

6.20; df = 1376; P < 0.001]). The tibia and wings of males reared on *B. lateralis* remained similar in size between Generations 1 and 2 (P > 0.05). The low sample size for Generation 3 limits its statistical use, but there was a notably large increase in the lengths of both body segments for female *A. hagenowii* reared on *B. lateralis* (P < 0.001) (Figs. 4.3 and 4.4). Generation 3 male *A. hagenowii* reared on *B. lateralis* were also larger compared to previous generations, but the change in size is less pronounced (P < 0.05) (Figs. 4.3 and 4.4).

Discussion

The results of this study indicate that *A. hagenowii* reared on *P. americana* will accept *B. lateralis* and *B. orientalis* as hosts with similar parasitization success. Female wasps reared from these hosts were significantly larger than those reared on *P. americana*. The oothecae of both *Blatta* species are typically longer (≈10.0 mm) than the oothecae of *P. americana* (≈ 8.0 mm) and may contain more nutrients to support the development of larger females (Schal 2011, Hedges 2021). During a preliminary study, 10 oothecae from each species were measured and *P. americana* oothecae were found to have a mean length of 7.6 mm and weight of 0.065 g, while the mean length and weight of *B. lateralis* oothecae was 9.0 mm and 0.089 g. Larger host size is typically correlated with increased parasitoid body size and fitness (Ueno 2015, Gao et al. 2016). However, declining trends in other fitness indicators (parasitism success and proportion female progeny) among *A. hagenowii* reared for multiple generations on *B. lateralis* and *B. orientalis* oothecae suggest that they may be poor long-term hosts (Henter 2003, Ueno 2015, Gao et al. 2016).

The reduced multi-generational performance of the *A. hagenowii* used in this study is likely due to their long history of laboratory rearing on only *P. americana* oothecae as hosts.

This strain originated from the colonies used by Pawson and Gold (1993) and were collected from the wild in 1991 from the oothecae of *P. americana* and its congener *P. fuliginosa* (Serville). The *A. hagenowii* reared on *P. fuliginosa* over the course of their experiment maintained a stable population and did not appear to have any loss of fitness. Furthermore, Pawson and Gold (1993) were able to successfully switch *A. hagenowii* from *P. americana* to *B. orientalis* for multi-generational rearing without any apparent impact on fitness. However, it is notable that both strains reared on *B. orientalis* and *P. fuliginosa* maintained a preference for *P. americana* oothecae in choice experiments (Pawson and Gold 1993). After 30 years of rearing on only *P. americana*, the descendants of the wild-caught *A. hagenowii* have been artificially selected to perform best on this host. Such selection pressure may have led to a loss in the ability to easily switch between alternative host species. Numerous studies have found that long-term mass rearing of parasitoids under laboratory conditions can negatively impact their fitness, ability to switch between hosts, and performance as biological control agents (Van Bergeijk et al. 1989, Ueno 2015, Bertin et al. 2017, Naranjo-Guevara et al. 2020).

It is difficult to determine which differences among the oothecae of each species contributed to the declines in fitness for *A. hagenowii*. Factors that may have affected parasitoid development include egg number, size, and nutrient composition, oothecal fluids, and endosymbiotic bacteria. Despite *Blatta* species producing longer and heavier oothecae than *P. americana*, all contain a similar number of eggs (≈ 16) on average (Roth and Willis 1954, Kim and Rust 2013; Personal Observations). The eggs of *B. lateralis* and *B. orientalis* may be larger than those of *P. americana* or have a different nutrient composition, but this has not been investigated. Likewise, there is very little information available about the nutrient quality of the fluid surrounding the eggs, which contains glucose and other molecules involved in the tanning

of the oothecal cuticle (Brunet and Kent 1955, Stay and Roth 1962). The embryos of the species used in this study each carry a unique strain of the endosymbiotic bacterium *Blattabacterium cuenoti* transmitted from their mother (Patiño-Navarrete et al. 2013, Noda et al. 2020). How these endosymbionts might affect *A. hagenowii* larvae, or the larvae of other parasitoids, is also unknown.

Based on our results with this strain, *A. hagenowii* used in an IPM program aimed at long lasting control of *B. lateralis* are likely to lose efficacy over time as female progeny and parasitism success decline with successive generations. However, declining efficacy can be addressed with periodic inundative releases and/or provisioning of *P. americana* oothecae. Repeated inundative releases over the course of several months are common practice when introducing *A. hagenowii* to an area for treatment (Pawson and Gold 1993, Suiter et al. 1998, Tee et al. 2011). Periodic inundative releases every two to three months (2 to 3 generations) after the introduction period is likely to boost the parasitoid population. Using wasps reared on *P. americana* in these releases would also introduce individuals unaffected by declines in fitness (i.e., reduced parasitism success and proportion female progeny) incurred by rearing on *B. lateralis*. After multiple generations on *B. lateralis*, the periodic inclusion of *P. americana* oothecae in the treatment area may provide an opportunity for *A. hagenowii* to switch to its preferred host and recover fitness losses. Tee et al. (2010) found that *A. hagenowii* would readily parasitize heat and cold-killed *P. americana* oothecae without detrimental effects on emergence, development time, or sex ratio. Thus, killed oothecae of the preferred host could be provided to released *A. hagenowii* without risking the introduction of additional pests.

As detailed by Pawson and Gold (1993), strains of *A. hagenowii* able to thrive on hosts other than *P. americana* are present in the wild. The wasps used to create their colonies came

from an area where at least three species of *Periplaneta* coexist (Atkinson et al. 1991, Pawson and Gold 1993). The *A. hagenowii* from this area were likely better adapted for parasitizing multiple host species, an ability that may have been lost as their descendants were selected for performance on *P. americana*. Wild parasitoids collected from similar areas with a greater cockroach diversity may more successfully switch to *B. lateralis* as a long-term host. Alternatively, suitable strains of *A. hagenowii* may exist in areas where *B. lateralis* is the predominant host species. In this case, selection would favor parasitoids that can maintain a sustainable population on *B. lateralis* oothecae alone. Lastly, the short-term success of *A. hagenowii* on *B. lateralis* and *B. orientalis* in the current study suggests that development of a strain better suited for long-term control is possible with current laboratory stock. Further testing may yield individuals that maintain fitness during host switching and are effective for *B. lateralis* suppression.

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References

Atkinson TH, Koehler PG, Patterson RS. Catalog and atlas of the cockroaches (Dictyoptera) of North America North of Mexico. Misc. Publ. Entomol. Soc. Am. 1991:78:1-86.

Bell HA, Marris GC, Edwards JP. The influence of the juvenile hormone analogue (S)-hydroprene on *Aprostocetus hagenowii* (Hymenoptera: Eulophidae), an oothecal parasitoid of the Oriental cockroach *Blatta orientalis* (Dictyoptera: Blattidae). Bull. Entomol. Res. 1998:88(3):231-238.

Bertin A, Pavinato VAC, Parra JRP. Fitness-related changes in laboratory populations of the egg parasitoid *Trichogramma galloi* and the implications of rearing on factitious hosts. Biocontrol 2017:62(4):435-444.

Bressan-Nascimento S, Oliveira DMP, Fox EGP. Thermal requirements for the embryonic development of *Periplaneta americana* (L.) (Dictyoptera: Blattidae) with potential application in mass-rearing of egg parasitoids. Biol. Control 2008:47(3):268-272.

Brunet PCJ, Kent PW. Observations on the mechanism of a tanning reaction in *Periplaneta* and *Blatta*. Proc. Royal Soc. B. 1955:144(915):259-274.

Campbell I. Chi-squared and Fisher-Irwin tests of two-by-two tables with small sample recommendations. Stat. Med. 2007:26(19):3661-3675.

Cueto-Medina SM, Castillo-Martínez A, Hernández-Rodríguez S, López RM, Sánchez-Ramos FJ, Ortega-Morales AI. Atlas fotográfico de las cucarachas del semidesierto Coahuilense, Mexico. Folia Entomol. Mex. 2015:2:767-775.

Davranoglou LR, Hadjiconstantis M, Mann DJ. First record of the Turkestan cockroach (*Shelfordella lateralis*) from Cyprus and Turkey (Dictyoptera: Blattidae). Isr. J. Entomol. 2020:50(1):1-8.

Deng W, Luo X, Ho SYW, Liao S, Wang Z, Che Y. Inclusion of rare taxa from Blattidae and Anaplectidae improves phylogenetic resolution in the cockroach superfamily Blattoidea. Syst. Entomol. 2023:48(1):23-39.

Donkor ES. Nosocomial pathogens: an in-depth analysis of the vectorial potential of cockroaches. Trop. Med. Infect. Dis. 2019:4(1):1-11.

Gaire S, Romero A. Comparative efficacy of residual insecticides against the Turkestan cockroach, *Blatta lateralis*, (Blattodea: Blattidae) on different substrates. Insects 2020:11(8):1-9.

Gao S, Tang Y, Wei K, Wang X, Yang Z, Zhang Y. Relationships between body size and parasitic fitness and offspring performance of *Sclerodermus pupariae* Yang *et* Yao (Hymenoptera: Bethylidae). PLoS One 2016:11(7):1-14.

Gondhalekar AD, Appel AG, Thomas GM, Romero A. A review of alternative management tactics employed for the control of various cockroach species (Order: Blattodea) in the USA. Insects 2021:12(6):1-20.

Goss-Sampson MA. Statistical analysis in JASP: a guide for students, 5th ed. 2022. (https://jasp-stats.org/wp-content/uploads/2022/04/Statistical-Analysis-in-JASP-A-Students-Guide-v16.pdf).

Hagenbuch BE, Koehler PG, Patterson RS, Brenner RJ. 1988. Peridomestic cockroaches (Orthoptera: Blattidae) of Florida: their species composition and suppression. J. Med. Entomol. 1988:25(5):377-380.

Harbison B. Turkestan cockroaches have made themselves at home in California. Valley View (OH): Pest Control Technology; 2022. [accessed 2022 Nov 20] (https://www.pctonline.com/article/turkestan-cockroach-explosion-california/).

Hebard M. Memoirs of the American Entomological Society number 2: the Blattidae of North America north of the Mexican boundary. Philadelphia (PA): American Entomological Society; 1917.

Hedges SA. Field guide for the management of structure-infecting cockroaches. Valley View (OH): GIE Media; 2021.

Henter HJ. Inbreeding depression and haplodiploidy: experimental measures in a parasitoid and comparisons across diploid and haplodiploid insect taxa. Evolution 2003:57(8):1793-1803.

Huang CF, Chie WC, Wang IJ. Effect of environmental exposures on allergen sensitization and the development of childhood allergic diseases: a large-scale population-based study. World Allergy Organ. J. 2021:14(1):1-12.

Kim T, Rust MK. Life history and biology of the invasive Turkestan cockroach (Dictyoptera: Blattidae). J. Econ. Entomol. 2013:106(6):2428-2432.

LeBeck LM. A review of the hymenopterous natural enemies of cockroaches with emphasis on biological control. Entomophaga 1991:36(3):335-352.

Miralles-Núñez A, Pradera C, Pérez-Gómez Á. New exotic cockroach established in the Iberian Peninsula? Confirmation of the presence of *Shelfordella lateralis* (Walker, 1986) (Blattodea: Blattidae) in the Iberian Peninsula and first records for Catalonia. Butll. Inst. Catalana Hist. Nat. 2020:84:203-205.

Naranjo-Guevara N, dos Santos LAO, Barbosa NCCP, e Castro ACMC, Fernandes OA. 2020. Long-term mass rearing impacts performance of the egg parasitoid *Telenomus remus* (Hymenoptera: Platygastridae). J. Entomol. Sci. 2020:55(1):69-86.

Narasimham AU. Comparative studies on *Tetrastichus hagenowii* (Ratzeburg) and *T. asthenogmus* (Waterston), two primary parasites of cockroach oothecae, and on their hyperparasite *Tetrastichus sp.* (*T. miser* (Nees) group) (Hymenoptera: Eulophidae). Bull. Entomol. Res.1984:74(2):175-189.

Narasimham AU. Comparative biological parameters of *Comperia merceti* (Compere) (Hym., Encyrtidae) and *Anastatus tenuipes* Bolivar (Hym., Eupelmidae), oothecal parasitoids of the cockroach *Supella longipalpa* (Fab.). Biol. Control. 1992:2(1):73-77.

Nasirian H. Contamination of cockroaches (Insecta: Blattaria) by medically important Bacteriae: a systematic review and meta-analysis. J. Med. Entomol. 2019:56(6):1534-1554.

Noda T, Okude G, Meng XY, Koga R, Moriyama M, Fukatsu T. Bacteriocytes and *Blattabacterium* endosymbionts of the German cockroach *Blattella germanica*, the forest cockroach *Blattella nipponica*, and other cockroach species. J. Zool. 2020:37(5):399-410.

Olson CA. *Blatta (Shelfordella) lateralis*, the Turkestan cockroach (Blattoidea: Blattidae) recorded from Arizona. Bull. Entomol. Soc. Am. 1985:31(2):30.

Patiño-Navarrete R, Moya A, Latorre A, Peretó J. Comparative genomics of *Blattabacterium cuenoti*: the frozen legacy of an ancient endosymbiont genome. Genome Biol. Evol. 2013:5(2):351-361.

Pawson BM, Gold RE. Evaluating different release strategies for the control of American cockroaches using the egg parasitoid, *Aprostocetus hagenowii*, In Wildey KB and Robinson WH, editors. Proceedings of the First International Conference on Urban Pests; Cambridge, UK.; 1993 p. 407-413.

Pomés, A, Glesner J, Calatroni A, Visness CM, Wood RA, O'Connor GT, Kattan M, Bacharier LB, Wheatly LM, Gern JE, Busse WW. Cockroach allergen component analysis of children with or without asthma and rhinitis in an inner-city birth cohort. J. Allergy Clin. Immunol. 2019:144(4):935-944.

Roth LM, Willis ER. The reproduction of cockroaches. Smithsonian Misc. Collect. 1954:122(12):1-49.

Roth LM, Willis ER. The biotic associations of cockroaches. Smithsonian Misc. Collect. 1960:141(1):1-440.

SAS Institute. Step-by-step programming with Base SAS 9.4, 2nd ed. Cary (NC): SAS Institute; 2015.

Shah SN, Fossa A, Steiner AS, Kane J, Levy JI, Adamkiewicz G, Bennett-Fripp WM, Reid M. Housing quality and mental health: the association between pest infestation and depressive symptoms among public housing residents. J. Urban Health 2018:95(5):691-702.

Schal C. Handbook of pest control: the behavior, life history, and control of household pests. 10th ed. In Hedges SA, editor. Chapter 2: Cockroaches. Richfield (OH): The Mallis Handbook Company; 2011. p. 151–290.

Smith CM, Chicas-Mosier AM, Appel AG, Fadamiro HY. Gravid *Periplaneta americana* (Blattodea: Blattidae) fails to detect or respond to the presence of the oothecal parasitoid *Aprostocetus hagenowii* (Hymenoptera: Eulophidae). Environ. Entomol. 2022:51(6):1086-1093.

Smith, LM II, Appel AG, Mack TP, Keever GJ, Benson EP. Comparative effectiveness of an integrated pest management system and an insecticidal perimeter spray for control of smokybrown cockroaches (Dictyoptera: Blattidae). J. Econ. Entomol. 1995:88(4):907-917.

Suiter DR, Patterson RS, Koehler PC. Seasonal incidence and biological control potential of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) in treehole microhabitats. Environ. Entomol. 1998:27(2):434-442.

Sumino T, Saito H, Ichioka H. Observance of the Turkistan [sic] cockroach, *Blatta lateralis* in a multi-floor warehouse in Aichi prefecture. Pestology 2006:21(1):13-15.

Tee HS, Saad AR, Lee CY. Suitability of heat- and freeze-killed oothecae of the American cockroach (Dictyoptera: Blattidae) as hosts for an oothecal parasitoid, *Aprostocetus hagenowii* (Hymenoptera: Eulophidae). J. Econ. Entomol. 2010:103(5):1770-1774.

Tee HS, Saad AR, Lee CY. Evaluation of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) for the control of American cockroaches (Dictyoptera: Blattidae) in sewers and crevices around buildings. J. Econ. Entomol. 2011:104(6):2031-2038.

Ueno T. Effects of host size and laboratory rearing on offspring development and sex ratio in the solitary parasitoid *Agrothereutes lanceolatus* (Hymenoptera: Ichneumonidae). Eur. J. Entomol. 2015:112(2):281-287.

[USDA] U. S. Department of Agriculture. A cockroach (*Blatta lateralis*) –California. Coop. Plant Pest Rep. Hyattsville (MD): USDA; 1978.

Van Bergeijk KE, Bigler F, Kaashoek NK, Pak GA. Changes in host acceptance and host suitability as an effect of rearing *Trichogramma maidis* on a factitious host. Entomol. Exp. Appl. 1989:52(3):229-238.

Tables and Figures

Table 4.1: Measurement results (least squares means) for *A. hagenowii* females and males reared on three hosts across three generations.

Host	Sex	N	Body Part LS Mean Length (mm) ± SEM	
			Hind Tibia	Forewing
P. americana	♀	479	0.59 ± 0.005	1.53 ± 0.009
	♂	221	0.51 ± 0.006	1.36 ± 0.011
B. lateralis	♀	472	0.64 ± 0.015	1.60 ± 0.025
	♂	395	0.52 ± 0.004	1.33 ± 0.007
B. orientalis	♀	434	0.65 ± 0.009	1.64 ± 0.006
	♂	452	0.54 ± 0.007	1.40 ± 0.005

Table 4.2: A comparison of *A. hagenowii* hind tibia and forewing lengths between sex and host. Values in bold indicate significantly different comparisons. Replicate number for each sex of wasp on each host: *P. americana* female = 479, male = 221; *B. lateralis* female = 472, male = 395; *B. orientalis* female = 434, male = 452).

Hosts	Sex	Body Part	
		Hind Tibia	Forewing
P. americana x *B. lateralis*	♀	t = 3.09; df = 1376; **P = 0.006**	t = 2.87; df = 1376; **P = 0.012**
	♂	t = 1.76; df =897; P = 0.184	t = -2.13; df = 943; P = 0.083
P. americana x *B. orientalis*	♀	t = 7.15; df = 1376; **P < 0.001**	t = 8.47; df = 1376; **P < 0.001**
	♂	t = 4.62; df = 909; **P < 0.001**	t = 3.18; df = 952; **P = 0.004**
B. lateralis x *B. orientalis*	♀	t = -0.57; df = 1376; P = 0.838	t = -1.45; df = 1376; P = 0.316
	♂	t = -3.99; df = 851; **P < 0.001**	t = -7.44; df = 889; **P < 0.001**

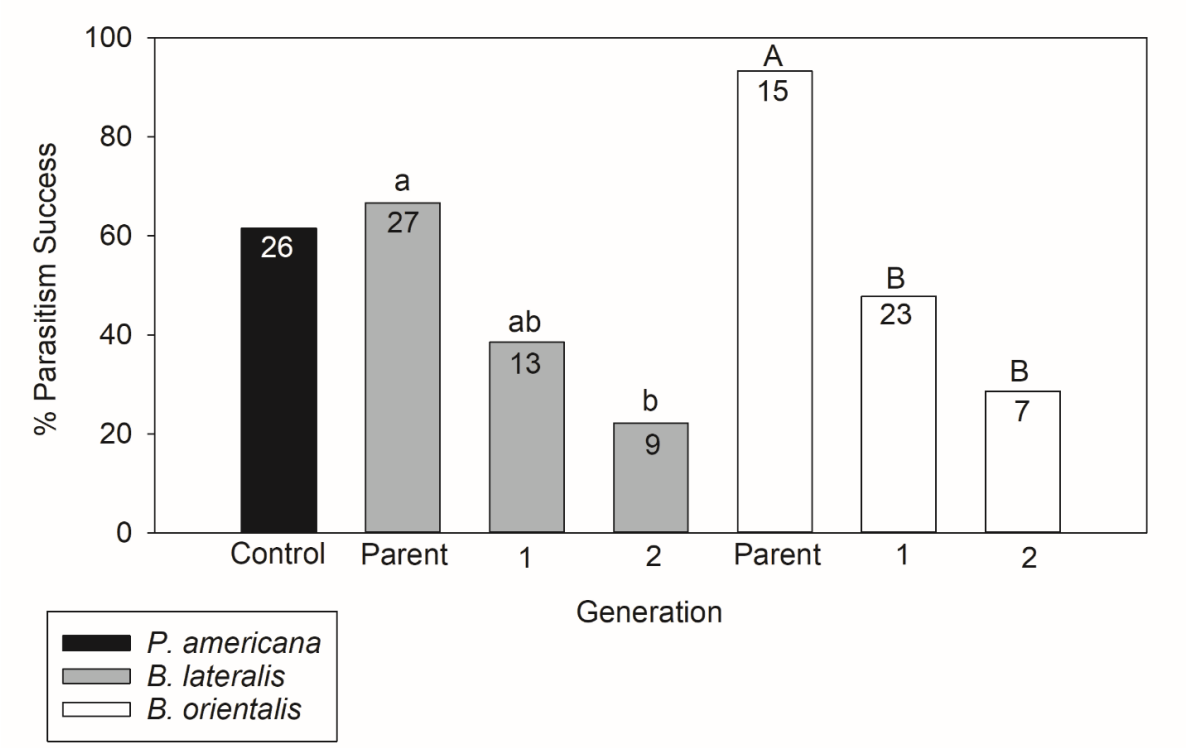

Figure 4.1: *Aprostocetus hagenowii* parasitization success of each generation of rearing on oothecae from *P. americana* (control), *B. lateralis*, and *B. orientalis*. Generational parasitism success was analyzed within host species. Replicate numbers are provided in each bar. Bars that do not share lowercase (*B. lateralis*) or uppercase (*B. orientalis*) letters are significantly different at P = 0.05.

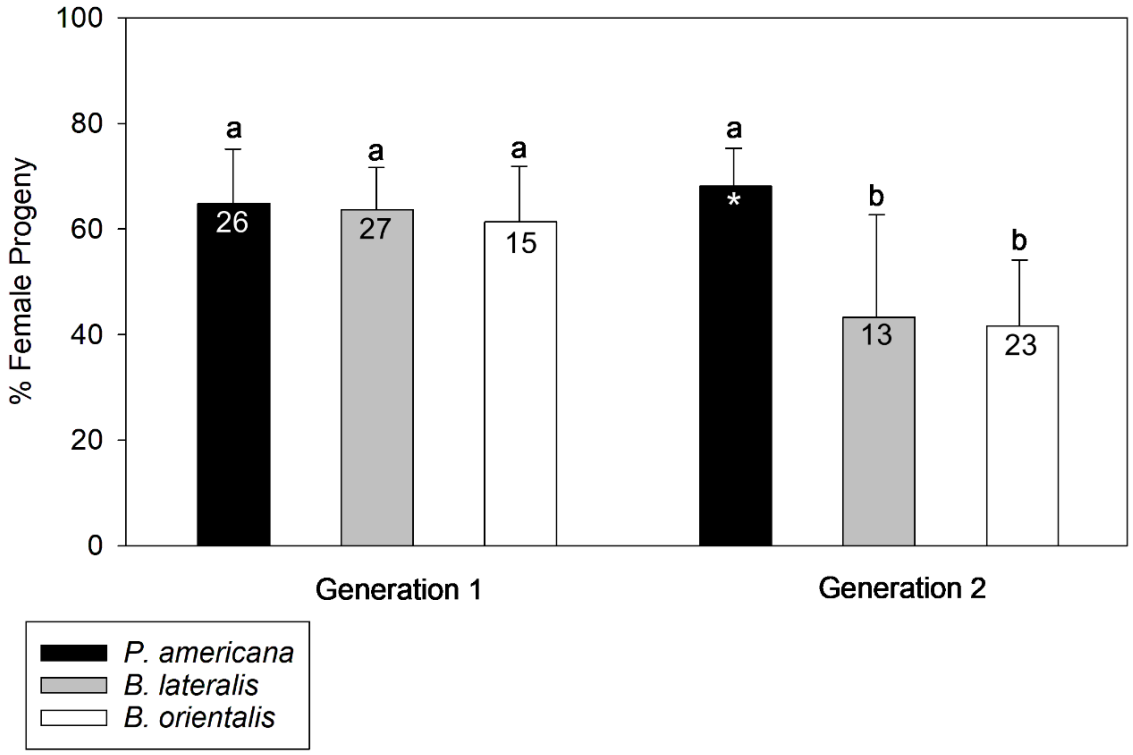

Figure 4.2: Comparison of the proportion female *A. hagenowii* wasps produced across two generations on three host species. Replicate numbers are provided in each bar. *Periplaneta americana* Generation 2 (*) was constructed using randomly selected wasps from *P. americana* Generation 1. Bars that do not share letters are significantly different at P = 0.05.

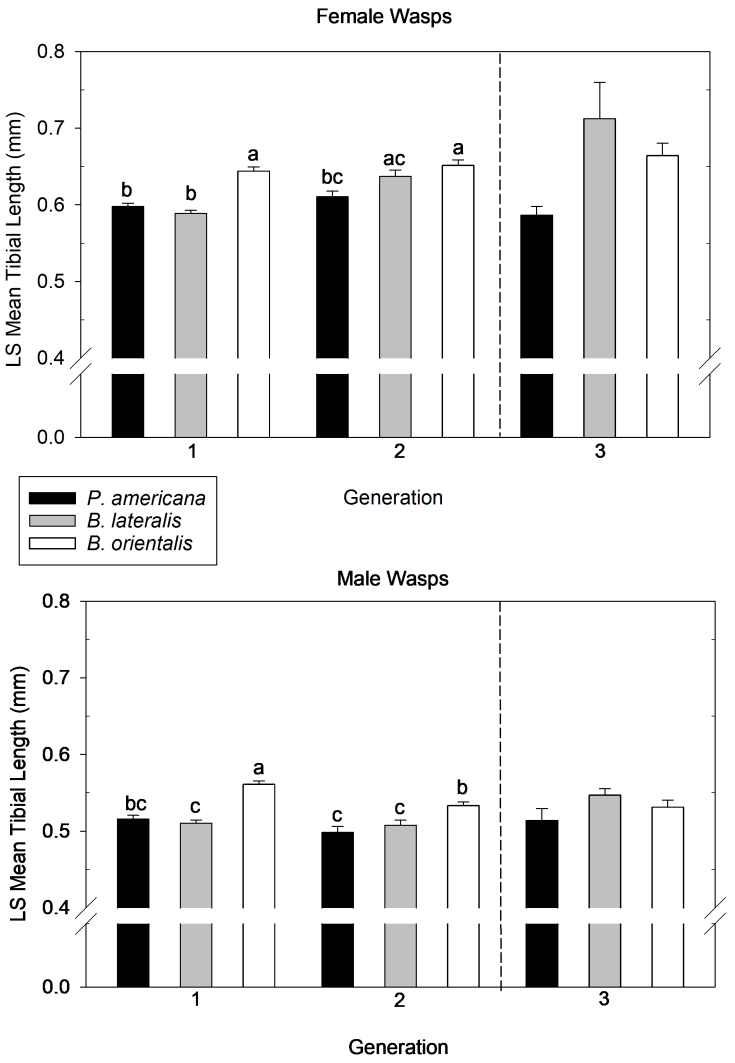

Figure 4.3: Comparison of mean (least-squares) female and male *A. hagenowii* tibia lengths across hosts and generations. Bars that do not share letters are significantly different at P = 0.05. Generation 3 is provided for comparison but without indication of significance due to low replicate number. Replicate number for each sex of wasp on each generation of host: *P. americana* Generation 1 female = 312, male = 148, Generation 2 female = 122, male = 58, Generation 3 female = 45, male = 15; *B. lateralis* Generation 1 female = 356, male = 250, Generation 2 female = 112, male = 85, Generation 3 female = 4, male = 60; *B. orientalis* Generation 1 female = 256, male = 224, Generation 2 female = 148, male = 180, Generation 3 female = 30, male = 48.

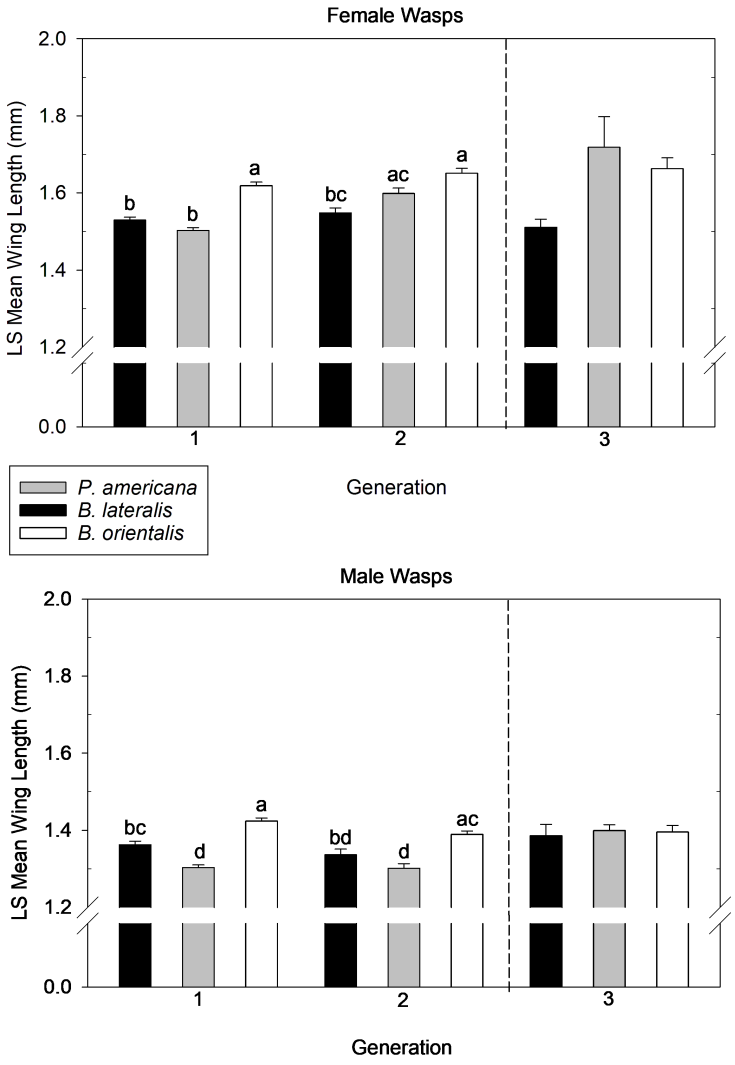

Figure 4.4: Comparison of mean (least-squares) female and male *A. hagenowii* wing lengths across hosts and generations. Bars that do not share letters are significantly different at $P = 0.05$. Generation 3 is provided for comparison but without indication of significance due to low replicate number. Replicate number for each sex of wasp on each generation of host: *P. americana* Generation 1 female = 312, male = 148, Generation 2 female = 122, male = 58, Generation 3 female = 45, male = 15; *B. lateralis* Generation 1 female = 356, male = 250, Generation 2 female = 112, male = 85, Generation 3 female = 4, male = 60; *B. orientalis* Generation 1 female = 256, male = 224, Generation 2 female = 148, male = 180, Generation 3 female = 30, male = 48.

Chapter 5: Toxicity of Cockroach Gel Baits to the Oothecal Parasitoid *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) and Implications for Cockroach IPM

Abstract

Aprostocetus hagenowii (Ratzeburg) is a parasitoid wasp that parasitizes the oothecae of peridomestic pest cockroaches. *Aprostocetus hagenowii* has been used in integrated pest management programs for cockroach control but there has been little research investigating how this wasp responds to the insecticides commonly used for cockroach management. Five insecticidal gel products (indoxacarb, clothianidin, fipronil, dinotefuran, and abamectin B1) were tested for their toxicity towards *A. hagenowii* and *Periplaneta americana* (L.), a host of *A. hagenowii* and a common pest. Each gel product was tested as a fresh and dried application. All products caused significant ($P < 0.05$) *A. hagenowii* mortality, except for indoxacarb, which had median survival times (168 h fresh, 72 h dry) and final mortalities (58.33% fresh, 95.83% dry) not significantly different from the control ($P > 0.05$). All products caused significant *P. americana* mortality as fresh and dry applications ($P < 0.05$). Notably, the dinotefuran product, which lost most of its weight (86.88%) after drying, lost much of its efficacy with final mortality dropping from 80% (fresh) to 32.24% (dry). There was no significant correlation ($P > 0.05$) between the responses of either species or within species to fresh or dry applications. The lack of correlation suggests that *A. hagenowii* and *P. americana* metabolize these insecticides in different ways and also indicates that drying affects each gel formulation in non-uniform ways. Indoxacarb appears most compatible for use alongside *A. hagenowii* in cockroach management programs.

Introduction

Aprostocetus hagenowii is a generalist parasitoid wasp that parasitizes the oothecae (egg cases) of cockroaches (Blattodea). Most known hosts for *A. hagenowii* are peridomestic pest species in the Blattidae, including the American cockroach *Periplaneta americana*, Australian cockroach *P. australasiae* (F.), and Oriental cockroach *Blatta orientalis* L. (LeBeck 1991). Numerous authors (Narasimham 1984, Hagenbuch et al. 1989, Pawson and Gold 1993, Suiter et al. 1998, Tee et al. 2011) have investigated the feasibility and best practices for using *A. hagenowii* as a biological control agent of pest cockroaches, particularly against its preferred host the American cockroach. Experimental releases of *A. hagenowii* in enclosed rooms, such as test kitchens typically achieve high (> 70%) parasitism rates (Roth and Willis 1954, Hagenbuch et al. 1989), but parasitism success was often much lower and varied widely during other types of field experiment: Narasimham (1984) reported 8% parasitism success in homes near outdoor inundation sites, Pawson and Gold (1993) found 2-44% parasitism in home pipe chases, and Tee et al. (2011) observed parasitism success rates of 13.3% and 18.1% in sewers and building crevices, respectively.

Aprostocetus hagenowii is unlikely to eradicate or provide adequate cockroach suppression when used as the only method of control. The inclusion of *A. hagenowii* within an integrated pest management (IPM) program is more likely to bring success, but this requires the use of additional and compatible treatment methods. Management of cockroaches typically involves the application of insecticidal chemicals in the form of sprays, baited gels, and solid baits (Schal 2011). However, very few studies have investigated how cockroach insecticides affect oothecal parasitoids. Only one study (Bell et al. 1998) has examined the direct effects (i.e., wasp mortality and physiological responses) of an insecticide, the insect growth regulator (S)-

hyrdoprene), on *A. hagenowii.* Studies by Hagenbuch et al (1989) and Rierson et al. (2005) have investigated using *A. hagenowii* alongside other insecticidal products, such as hydramethylnon, fipronil, and imidacloprid, but only the indirect effects (i.e., parasitism success) were monitored. The studies by Hagenbuch (1989) and Rierson (2005) found the insecticides to have no effect on parasitism success. However, Bell et al. (1998) noted that (S)-hydroprene caused wing deformities and decreased parasitism success, both of which would limit dispersal and hinder long-term control of hosts.

The objective of this study was to test a variety of cockroach insecticidal gel products (Table 5.1) for their compatibility with *A. hagenowii*. *Periplaneta americana* was selected for concurrent testing of the gel products as it is the preferred host of *A. hagenowii* and a common peridomestic pest species (Pawson and Gold 1993, Schal 2011). Each product was expected to cause *A. hagenowii* mortality, especially those marketed for both cockroach and ant control. Products that caused limited *A. hagenowii* mortality and high cockroach mortality would be considered most compatible for use alongside the parasitoid for cockroach control.

Methods and Materials

Toxicity Tests on *Aprostocetus hagenowii*

The *A. hagenowii* used in this study were obtained from Dr. Barry Pawson of PNE, Inc (Tipp City, OH; retired) and have been reared at Auburn University since 2020. The colony was reared according to Smith et al. (2022) (Chapter 1) in 100 x 15 mm polystyrene Petri dishes on the oothecae of *P. americana*. Parasitoids were incubated within a growth chamber at 25 ± 2°C, 30–40% RH, and a 16:8 (L:D) photoperiod. Upon emergence from host oothecae, wasps were

released into a stinging cage and afforded a 24 h mating period before their use in experiments. It is unclear what effect feeding or starvation status may have on the toxicity of the products tested on *A. hagenowii*. Thus, the wasps were also provided with short pieces of dental wick that had been soaked in a honey-water solution at least 6 h before the start of each experiment. The honey-water solution was created by dissolving two to three large drops (approx. 1-2 mL) of raw honey (Member's Mark Bee Proud White Honey, Sam's Club, Bentonville, AR) in 30 mL of warm water in the lower half of a 100 x 15 mm Petri dish. The soaked wicks were placed throughout the stinging cage so that wasps could feed *ad libitum* according to individual needs.

Five gel bait formulations manufactured for cockroach control were tested: Advion® (Syngenta, Basel, Switzerland), Maxforce® Impact™ (Bayer AG, Leverkusen, Germany), Combat® Max™ (Henkel Corp., Rocky Hill, CT), Hot Shot® (United Industries Corp., St. Louis, MO), and Vendetta® (MGK Co., Minneapolis, MN). Information about the active ingredients and characteristics of each gel is provided in Table 5.1. Separate experiments were conducted with fresh gel and 24-hour-old, dried gel to determine the effect that age and water content had on toxicity. An experimental replicate consisted of a 35 x 15 mm polystyrene Petri dish containing 6 *A. hagenowii* wasps of random sex and a small piece (1 x 1 cm) of filter paper holding a drop of cockroach gel (approx. 0.02 g) or a piece (1 x 1 x 0.5 cm) of dental wick wetted with 2-3 drops of water (control). The mean dose of each gel applied during experiments is provided in Table 5.1. Applicator size and gel consistency account for non-uniformity in dose sizes between products. To test the toxicity of dried bait for wasps and cockroaches, fresh bait was applied to filter paper as above and dried at 22 ± 2 °C and 40-50% RH for 24 in a screened cage (BugDorm-1, MegaVeiw Science Co, Taiwan). The screened cage provided airflow over the drying gels while also keeping insects from feeding on the gels as they dried. A piece of filter

paper absent gel and water was provided to wasps in the dried gel control group. The room used during experiments was maintained at 22 ± 2 °C, 40-50% RH, and under constant light to simulate the environment within a home or business. During testing of the gels, wasp behavior and mortality were recorded at the 5, 15, 30, 60, 120, and 180 min time points, then checked once every 24 h until at least 72 h had passed. *Aprostocetus hagenowii* has a short adult lifespan and is sensitive to water loss. Thus, 2-3 drops of water were applied to wicks and filter paper in the fresh gel treatment group at 72 h and at subsequent 48 h time periods until the majority of the wasps had died. Most of the wasps in the dried gel experiment were dead by 72 h. Dead wasps were left *in situ* over the course of experiments.

To determine the water content of each gel formulation, 6 samples (approx. 0.02 g) of each gel were placed in open, individual 35 x 15 mm polystyrene Petri dishes, weighed, and dried for 24 h in a screened insect cage. The gels were reweighed, then the open dishes were transferred to an oven at 46 °C. A sample from each gel treatment was weighed every 2 days to monitor water loss. After 8 days the gels ceased to lose weight and their final weights were recorded.

Toxicity Tests on *Periplaneta americana*

The *P. americana* used in this study were collected from colonies reared at Auburn University since 1985 and have never been exposed to insecticides. Colonies were maintained in 121.1 L (32 gal) plastic garbage bins (Rubbermaid, Atlanta, GA) containing individuals of mixed sex and age. Rolled and stapled tubes of corrugated cardboard were provided as harborage, and a diet of Purina Laboratory Diet 5001 rat chow blocks, Purina Dog Chow Complete Adult chicken flavor

formula (Ralston Purina, St. Louis, MO), and water was provided *ad libitum*. The cockroach colony room was maintained at 27 ± 2°C, 45–50% RH, and a 12:12 (L:D) photoperiod.

Each replicate consisted of 6 adult male *P. americana* held in a 0.94 L (1 qt) wide-mouth glass jar. Adult male cockroaches were selected for this experiment because they have less variation in physiological state compared to immatures and adult females. Each jar was stocked with a 5 cm x 9 cm piece of cardboard as harborage. A piece of dog chow and a wetted dental wick were provided as competitive sources of food and water. A thin band of petroleum jelly was also applied to the inner, upper 5 cm of the jar to prevent escape, and a piece of filter paper held in place by a canning jar ring was used as a lid. The cockroaches in each replicate were exposed to one of 5 gel products (Table 5.1) or the control; There were 6 replicates per treatment. Replicates were grouped in a randomized complete block design to account for the potential effects that jar position may have on light, temperature, etc. Each gel was administered as an approx. 0.5 g dose on a plastic weigh boat, while control replicates were provided with an empty weigh boat. This experiment was conducted under the same environmental conditions as the wasp experiment. Cockroach behavior and mortality were recorded at the 5, 15, 30, 60, 120, and 180 min time points, then checked twice every 24 h until 165 h. Additional food, water, and gel were not provided after the start of the experiment. Dead cockroaches were left *in situ* throughout the course of the experiment. A second toxicity test was carried out with *P. americana* under the same methods and conditions except that each gel product was air dried for 24 h before exposure to the cockroaches.

Statistical Analysis

Survivorship of wasps and cockroaches was analyzed using Gehan-Breslow survivorship (Etikan et al. 2018) and Bonferroni post-hoc analyses in SigmaPlot 13 (Systat Software, Inc., San Jose, CA). Median survival time (MST, time point at which half of the population remains alive) was also analyzed in SigmaPlot 13. Median lethal time (LT_{50}, time point with half of the population dead) was determined using probit analysis in Polo Plus (LeOra Software LLC, Parma, MO). SigmaPlot 13 was used to conduct Pearson correlation analyses of LT_{50} results. Two-tailed Z-tests were used for comparative analysis of percent final mortality (i.e., the proportion of wasps or cockroaches dead at the end of each experiment). Statistical results were compared to a threshold of $\alpha = 0.05$ for survivorship, Pearson correlation, and Z-test analyses. Comparisons between LT_{50} values were considered significantly different if there was no overlap in 95% confidence intervals (CI). Data are described as means with 95% CI or ± SEM where appropriate. Survivorship plots were generated using the Kaplan-Meier package in SRPlot (https://www.bioinformatics.com.cn/en) an online platform for data analysis and visualization.

Results

Toxicity Tests on *Aprostocetus hagenowii*

Median survival time for the control group in the fresh gel experiment was 192 h (CI = 163.29-220.70) and the final mortality was 66.67% (N = 24). Compared to the control, fresh applications of the Maxforce Impact, Combat Max, and Hot Shot gel formulations had significantly negative ($P \leq 0.001$) impacts on the survival of *A. hagenowii* (Table 5.2). Median survival times were ≤ 48 h, and final mortality was > 85.0% for these three treatments (Table 5.2). However, only

Maxforce Impact and Combat Max had significantly higher final mortality ($P < 0.01$) (Table 5.2). The fresh Advion and Vendetta treatment groups had MSTs of ≥ 120 h and did not cause significant ($P > 0.05$) impacts on survival time compared to the control (Table 5.2). The final mortality for Advion was lower than the control (58.33%), and the final mortality for Vendetta was higher (75.00%), but neither were significantly different from the control ($P > 0.05$). The LT_{50} values for Maxforce Impact (19.99 h, CI = 14.91-25.79 h), Combat Max (28.22 h, CI = 23.23-32.85 h), and Hot Shot (11.72 h, CI = 8.20-16.77 h) were each significantly lower (i.e., the 95% CI intervals do not overlap) than the control (200.64 h, CI = 170.49-262.52 h) and under 30 h, which indicates rapid mortality. The LT_{50} value for Vendetta (113.83 h, CI = 91.33-148.27 h) was more than 3x higher than Maxforce, Combat, and Hot Shot but still significantly less than the control. Advion (171.20 h, CI = 146.44-214.71 h) was the only treatment not significantly different from the control. Survival curves for freshly applied gels are displayed as Figs. 5.1 and 5.2.

The MST for the control group in the dried gel experiment was 96 h (CI = 74.03-117.97) and final mortality was 86.36%. Dried applications of Combat Max and Maxforce Impact had the same MST and similar final mortality as their freshly applied counterparts (Table 5.3). Dried Maxforce Impact was particularly fast acting compared to fresh and had a significantly low LT_{50} of 3.53 h (CI = 2.38-5.05 h) (Table 5.2). Vendetta appears to have become more toxic after drying as median survival and LT_{50} times dropped to 24 h (CI = 10.49-37.51 h) and 22.88 h (CI = 14.15-37.74 h), respectively (Table 5.3). Final toxicity for Vendetta was similar between fresh and dry, however (Table 5.3). The survival curve and LT_{50} for Combat Max, Maxforce, and Vendetta were all significantly ($P < 0.01$) lower than the control. Dried Advion caused higher mortality (95.83%), lower MST, curve, and LT_{50} (< 72 h), but these changes were not

significantly different from the control ($P > 0.05$) (Table 5.3). Dried Hot Shot appeared to have lost potency compared to fresh applications (Table 5.3). The MST increased to 96 h (CI = 82.79-109.21 h), and LT_{50} increased to 66.19 h (CI = 57.71-74.38 h), both values were not significantly different from the control ($P > 0.05$). Final mortality for dried Hot Shot increased by < 10% compared to the fresh application (Table 5.3). Survivorship curves for dried gels are displayed as Figs. 5.3 and 5.4. There was no significant correlation between the LT_{50} values of *A. hagenowii* exposed to fresh and dried gels ($r = 0.314$; $P = 0.607$).

Toxicity Tests on *Periplaneta americana*

During the fresh gel experiment, the control treatment group had no mortalities, and all the gel products had a significantly ($P < 0.01$) negative impact on survival time when compared to the control group (Table 5.6). Advion, Combat, and Hot Shot caused rapid mortality early in the experiment (MST all = 40 h). However, the survivorship curves (Figs. 5.5 and 5.6) show marked differences in how the treatments affected survival over time. The Combat and Hot Shot curves show sharp, early declines (LT_{50} = 29.34 h, CI = 17.15-39.59 h and LT_{50} = 25.86 h, CI = 16.91-38.14 h, respectively), but Hot Shot lost effectiveness and its curve flattened while the Combat curve continued its downward trend (Fig. 5.5). Advion had a delayed effect followed by a sharp decline in survival (LT_{50} = 41.93 h, CI = 32.25-50.91 h). Maxforce (MST = 65 h; LT_{50} = 45.63 h, CI = 29.20-66.59 h) and Vendetta (MST = 118 h; LT_{50} = 108.47 h, CI = 97.27-123.57 h) were much slower to act but caused steady mortality from the 12 h and 40 h marks, respectively (Figs. 5.5 and 5.6). The final mortalities for all gel products were high (74-100%) (Table 5.6). Combat gel was significantly more effective ($P < 0.01$) than all other fresh gel applications, except Hot Shot ($P = 1.00$), and Vendetta was the least effective ($P > 0.05$) (Table 5.8).

The control group of the dried gel experiment had only a single mortality (2.78%), and all the gel products had a significantly ($P < 0.01$) negative impact on MST compared to the control (Table 5.7). Maxforce was the fastest acting (MST = 18 h; LT_{50} = 17.95 h, CI = 13.78-22.47 h) among the dried gels followed by Combat (MST = 42 h; LT_{50} =32.64 h, CI = 25.69-38.99 h) and Advion (MST = 48 h; LT_{50} = 42.74 h, CI = 39.08-46.16 h) (Tables 5.7 and 5.9). Dried Vendetta had an MST of 145 h (LT_{50} = 137.73 h, CI = 127.16-153.21 h), while dried Hot Shot (Mean survival time = 144 h; LT_{50} = 401.94 h, CI = 250.93-1143.56 h) was the slowest acting overall (Tables 5.7 and 5.9). Dried Advion (100%), Maxforce (88.89%), and Combat (97.14%) caused high final mortalities. Dried Vendetta (54.29%) and Hot Shot (32.24%) caused much lower final mortalities. Pearson correlation analyses of LT_{50} data found no significant correlation in the responses of *P. americana* to fresh and dried applications of gel (r = -0.134, P = 0.830). There was also no correlation between the responses of *A. hagenowii* and *P. americana* to either the fresh (r = 0.448, P = 0.449) or dried (r = 0.586, P = 0.299) applications of the gels.

Discussion

Advion appears to be the insecticidal gel most compatible for use alongside *A. hagenowii* in an IPM program for cockroach control. Both fresh (MST = 168 h, LT_{50} = 171.20 h) and dried (MST = 72 h, LT_{50} = 63.07 h) Advion applications were among the slowest acting treatments for *A. hagenowii*, while being among the fastest acting against *P. americana* (Fresh: MST = 40 h, LT_{50} = 41.93; Dried: MST = 48 h, LT_{50} = 42.74 h). The final mortality for *A. hagenowii* exposed to freshly applied Advion was 58.33%, and the final mortality for *P. americana* was 97.22%. Wasps in the dried Advion treatment group were nearly all (95.83%) dead by the end of the experiment (120 h). However, the final mortality for dried Advion was not significantly different from the

control (86.36%; 1.139, $P = 0.254$). The other gel products are less compatible for use with *A. hagenowii* because they had significantly lower survival curves and LT_{50} times ($P < 0.05$) for the wasps as fresh (Maxforce, Hot Shot, and Combat) and/or as dried (Maxforce, Combat, Vendetta) applications compared to their respective controls (Tables 5.2 and 5.3).

Aprostocetus hagenowii adults held without food or water live < 4 d on average (Narasimham 1984). The wasps held in the dry control treatment group spent 5 d under starvation and dehydration conditions before the experiment was ended due to high mortality (> 80%) in the control group. Comparatively, the wasps held in the fresh control group were periodically provided with water and the experiment was able to continue for 9 d. By the 72 h (3 d) mark of the dried gel experiment, *A. hagenowii* mortalities among the Advion and control treatment groups were 50.00% and 40.90%, respectively, which are not significantly different from each other ($z = 0.618$, $P = 0.535$) but are significantly different from their final mortalities at the 120 h mark (Advion: $z = 3.573$, $P = 0.004$; Control: $z = 3.134$, $P = 0.002$). Dried Hot Shot was the only other product with a mortality (45.83%) at 72 h that was significantly lower than the final mortality (95.83%; $z = 3.811$, $P < 0.001$), which indicates that mortality in the Maxforce, Combat, and Vendetta treatment groups was primarily due to their active ingredients, not from starvation or dehydration. Hot Shot was also the only product to lose toxicity (MST and LT_{50} times increased) when provided as a dry application for the wasps (Tables 5.2 and 5.3). Compared to all the other gel products, Hot Shot had the highest proportion of water content lost after drying for 24 h (Table 1). Water loss may render Hot Shot gel less attractive, palatable, or effective for the delivery of its active ingredient (dinotefuran). However, studies (Appel 1992, Appel and Benson 1995, Oz et al. 2010) examining the effects of aging and water loss on other gel products did not find negative impacts to their toxicity for German cockroaches (*Blattella*

germanica L.). Further research is needed to determine if water loss, species preference, or another factor associated with gel aging caused reduced Hot Shot gel toxicity for *P. americana*.

Both species were observed to repeatedly feed on and make contact with the gels, indicating that they are not repellent to *A. hagenowii* or *P. americana*. Several individuals of both species were also observed to die after making contact, without feeding, on the gels. The absence of a significant correlation between the responses of the two species to the gels suggests that there are differences in how the pesticides may enter their bodies and/or undergo detoxification. Likewise, the lack of a correlation between each species' responses to fresh and dry applications of the same products indicates that the effects of gel aging are not uniform across products. For example, the proportion of water lost in each gel differed after drying for 24 h (Table 5.1). While the gel products used during this study are marketed primarily for cockroach control, their insecticidal active ingredients are also used in formulations targeting hymenopteran pests, primarily ants (e.g., Sumari® ant gel bait, MGK; Hot Shot® ultra liquid ant bait, United Industries) (Rice and Silverman 2012). Indoxacarb, which is effective against fire ants (*Solenopsis invicta* Buren) (Oi and Oi 2006), is a pro-insecticide that requires metabolic conversion into its active form *N*-decarbomethoxyllated JW062 (DCJW) before it can act as a sodium channel blocker (Wing et al. 1998, Wolfe and Scharf 2022). Differences in behavior and the expression of the genes controlling detoxification, among *A. hagenowii, S. invicta,* and *P. americana* may explain why *A. hagenowii* survives exposure to indoxacarb while the other two species are killed. *Solenopsis invicta* workers that first ingest indoxacarb partially digest the bait formulation before feeding their gut contents to other members of the colony via trophallaxis (Oi and Oi 2006). Colony members that feed on partially digested indoxacarb receive the active, toxic form of the insecticide (Oi and Oi 2006). Cockroaches are also known to feed on their

vomit and the vomit of other individuals (Buczkowski et al. 2008). *Aprostocetus hagenowii* is not a social wasp and does not display such feeding behaviors, which likely reduces secondary exposure to indoxacarb's toxic form. It is unclear what enzymes are responsible for the conversion (Wolfe and Scharf 2022), but the families of genes that control the expression of detoxification enzymes are diverse across insect taxa (Dermauw et al. 2020). *Aprostocetus hagenowii* may also lack the genes necessary for its metabolic pathways to quickly or fully produce DCJW.

Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands) sells numerous species of parasitoid wasps for biological control and provides a side effects tool (https://sideeffects.koppert.com/side-effects) categorizing insecticides by the severity of each's effects on their parasitoid products. Koppert Biological Systems sells wasps from the families Eulophidae, Aphelinidae, Braconidae, and Trichogrammatidae. The side effects tool lists indoxacarb spray applications as being "harmless or slightly harmful (< 25% reduction)" to "moderately harmful (25-50% reduction)" for the "control capacity" of adult wasps in these families. The same tool lists all the other insecticides used in this study as causing "harmful (50-75% reduction)" to "very harmful (> 75% reduction)" effects for the same wasp species as well. The information provided by the side effects tool suggests that wasps in these 4 families, or at least in the encompassing Chalcidoidea and Ichneumonoidea superfamilies, may be tolerant to the effects of indoxacarb. However, citations and detailed methodology are not provided, and the species available in the side effects tool are too few in number (e.g., one species in Eulophidae, 3 species in Aphelinidae), to adequately represent their respective families. If indoxacarb is compatible with these wasp families, there is potential for the creation of IPM plans that can

include insecticides alongside hymenopteran biological control agents without concerns over those agents losing efficacy.

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References Cited

Appel AG. Performance of gel and past bait products for German cockroach (Dictyoptera: Blattellidae) control: laboratory and field studies. J. Econ. Entomol. 1992:85(4):1176-1183.

Appel AG, Benson EP. Performance of abamectin bait formulations against German cockroaches (Dictyoptera: Blattellidae). J. Econ. Entomol. 1995:88(4):924-931.

Bell HA, Marris GC, Edwards JP. The influence of the juvenile hormone analogue (S)-hydroprene on *Aprostocetus hagenowii* (Hymenoptera: Eulophidae), an oothecal parasitoid of the oriental cockroach *Blatta orientalis* (Dictyoptera: Blattidae). Bull. Entomol. Res. 1998:88(3):231-238.

Buczkowski G, Scherer CW, Bennet GW. Horizontal transfer of bait in the German cockroach: indoxacarb causes secondary and tertiary mortality. J. Econ. Entomol. 2008:101(3):894-901.

Dermauw W., Van Leeuwen T, Feyereisen R. Diversity and evolution of the P450 family in arthropods. Insect Biochem. Mol. Biol. 2020:127:1-22.

Etikan I, Bukirova K, Yuval M. Choosing statistical tests for survival analysis. Biom. Biostat. Int. J. 2018:7(5):477-481.

Hagenbuch BE, Patterson RS, Koehler PG. Biological control of the American cockroach (Orthoptera: Blattidae) with inundative releases of *Tetrastichus hagenowii* (Hymenoptera: Eulophidae). J. Econ. Entomol. 1989:82(1):90-94.

LeBeck LM. A review of the hymenopterous natural enemies of cockroaches with emphasis on biological control. Entomophaga 1991:36(3):335-352.

Narasimham AU. Comparative studies on *Tetrastichus hagenowii* (Ratzeburg) and *T. asthenogmus* (Waterston), two primary parasites of cockroach oothecae, and on their hyperparasite *Tetrastichus sp.* (*T. miser* (Nees) group) (Hymenoptera: Eulophidae). Bull. Entomol. Res.1984:74(2):175-189.

Oi DH, Oi FM. Speed of efficacy and delayed toxicity characteristics of fast-acting fire ant (Hymenoptera: Formicidae) Baits. J. Econ. Entomol. 2006:99(5):1739-1748.

Oz E, Cetin H, Cilek JE, Devect O, Yanikoglu A. Effects of two temperature storage regimes on the efficacy of 3 commercial gel baits against the German cockroach, *Blattella germanica* L. (Dictyoptera: Blattellidae). Iran. J. Public Health. 2010:39(3):102-108.

Pawson BM, Gold RE. Evaluating different release strategies for the control of American cockroaches using the egg parasitoid, *Aprostocetus hagenowii*, In Wildey KB and Robinson WH, editors. Proceedings of the First International Conference on Urban Pests. Exeter (UK): BPCC Wheatons Ltd; 1993 p. 407-413.

Reierson DA, Rust MK, Paine E. Control of American cockroaches (Dictyoptera: Blattidae) in sewer systems. In Lee CY and Robinson WH, editors. Proceedings of the Fifth International Conference on Urban Pests; P & Y Design Network, Penang, Malaysia; 2005 p. 141-148.

Rice ES, Silverman J. Efficacy of insecticide baits against Asian needle ants, 2011. Arthropod Manag. Tests 2012:37(1):1

Roth LM, Willis ER. The biology of the cockroach egg parasite, *Tetrastichus hagenowii* (Hymenoptera: Eulophidae). Trans. Am. Entomol. 1954:80(2):53-72.

Schal C. Handbook of pest control: the behavior, life history, and control of household pests. 10th ed. In Hedges SA, editor. Chapter 2: Cockroaches. Richfield (OH): The Mallis Handbook Company; 2011. p. 151–290.

Suiter DR, Patterson RS, Koehler PC. Seasonal incidence and biological control potential of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) in treehole microhabitats. Environ. Entomol. 1998:27(2):434-442.

Tee HS, Saad AR, Lee CY. Evaluation of *Aprostocetus hagenowii* (Hymenoptera: Eulophidae) for the control of American cockroaches (Dictyoptera: Blattidae) in sewers and crevices around buildings. J. Econ. Entomol. 2011:104(6):2031-2038.

Wing KD, Schenee ME, Sacher M, Connair M. A novel oxadiazine insecticide is bioactivated in Lepidopteran larvae. Arch. Insect Biochem. Physiol. 1998:37(1):91-103.

Wolfe ZM, Scharf ME. Microbe-mediated activation of indoxacarb in German cockroach (*Blattella germanica* L.). Pestic. Biochem. Phys. 2022:188:1-9.

Figures and Tables

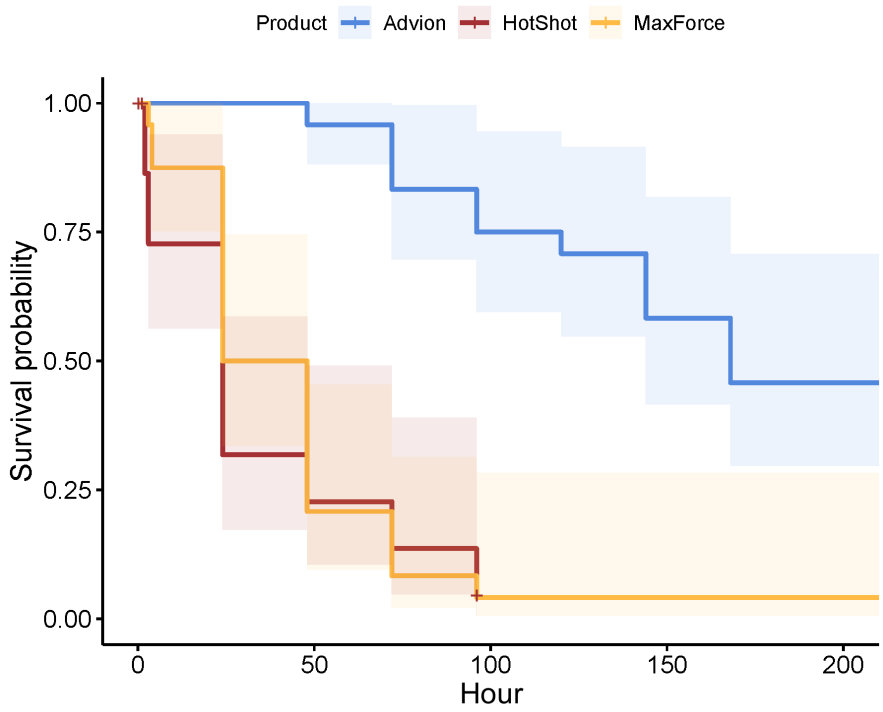

Figure 5.1. Survivorship trends for *A. hagenowii* exposed to freshly applied Advion, Hot Shot, and Maxforce cockroach insecticidal gel treatments.

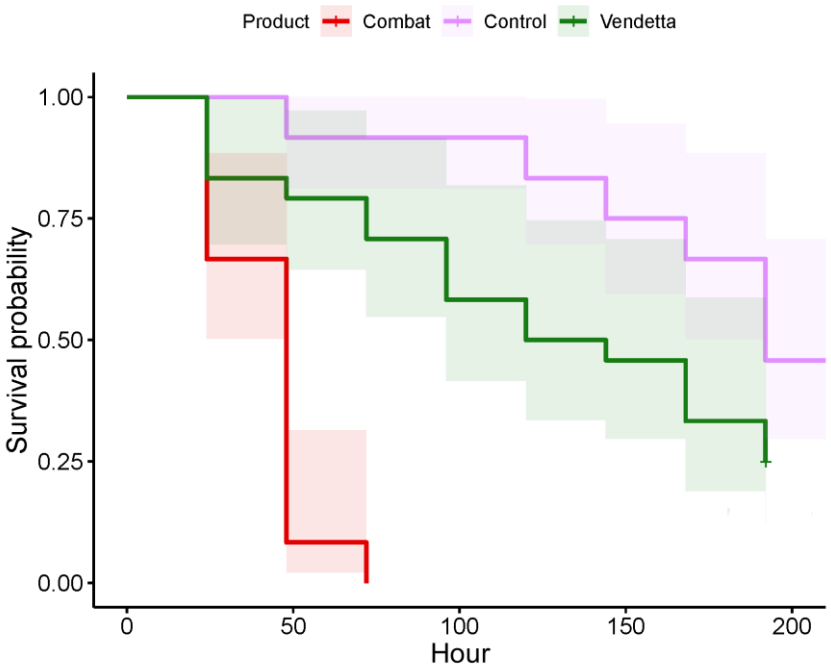

Figure 5.2. Survivorship trends for *A. hagenowii* exposed to freshly applied Combat and Vendetta cockroach insecticidal gel treatments as well as the control (water wick).

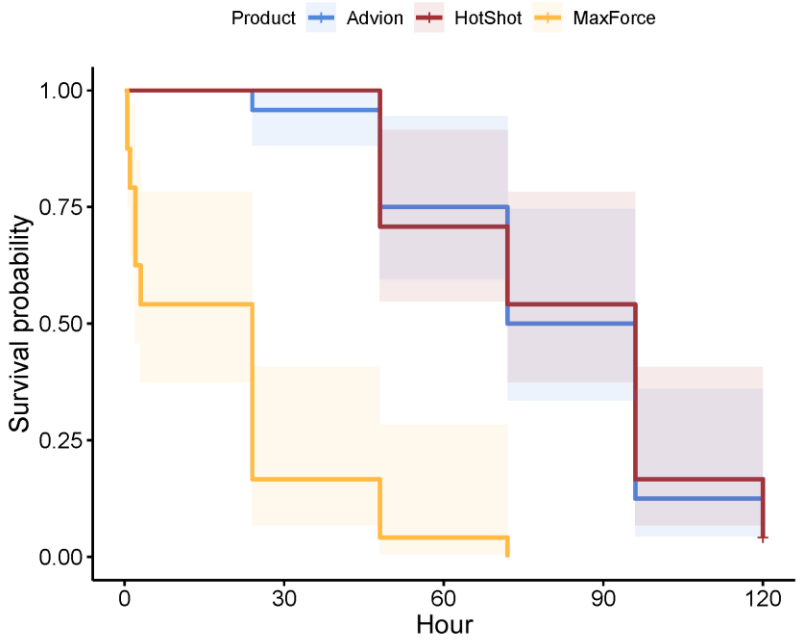

Figure 5.3. Survivorship trends for *A. hagenowii* exposed to dried applications of Advion, Hot Shot, and Maxforce cockroach insecticidal gel treatments.

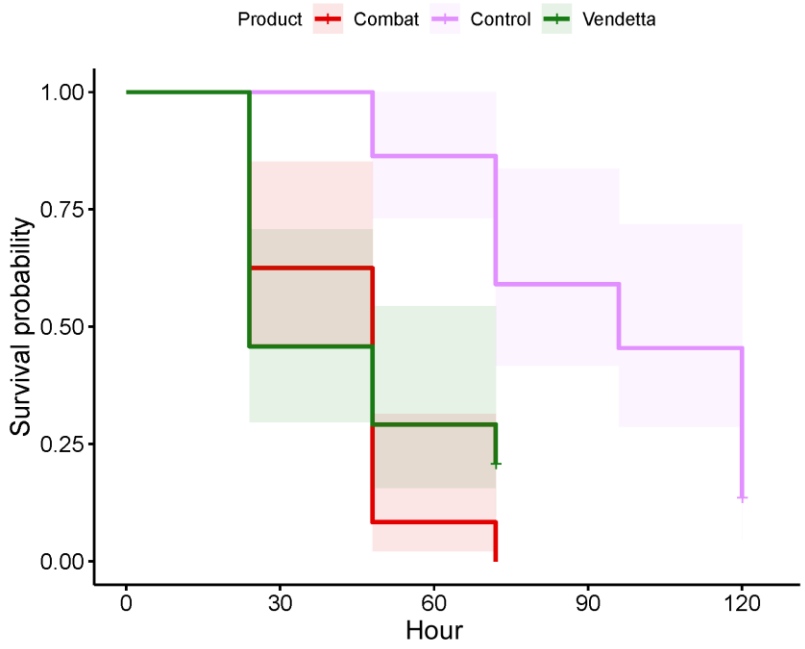

Figure 5.4. Survivorship trends for *A. hagenowii* exposed to dried applications of Combat and Vendetta cockroach insecticidal gel treatments as well as the control (dry filter paper).

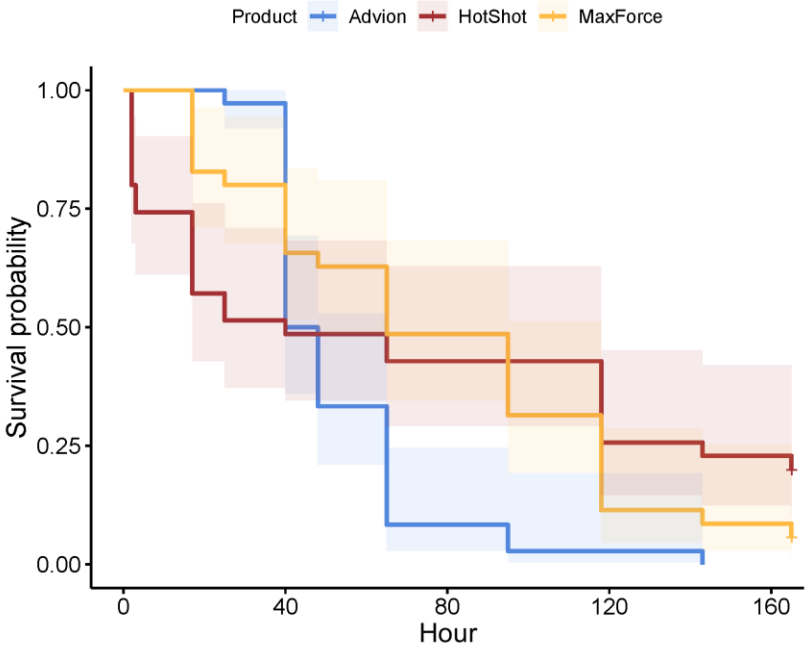

Figure 5.5. Survivorship trends for *P. americana* exposed to fresh applications of Advion, Hot Shot, and Maxforce cockroach insecticidal gel treatments.

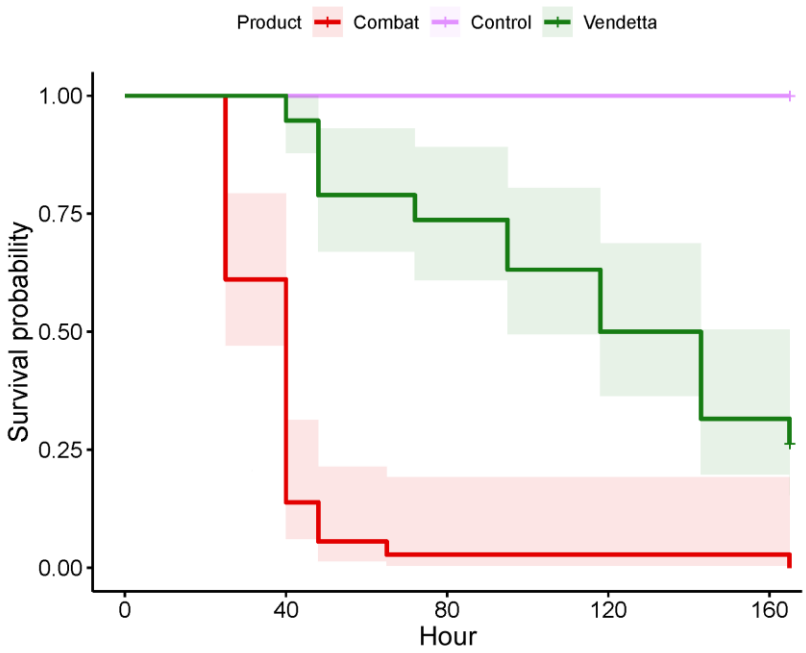

Figure 5.6. Survivorship trends for *P. americana* exposed to fresh applications of Combat and Vendetta cockroach insecticidal gel treatments as well as the control (empty weigh boat).

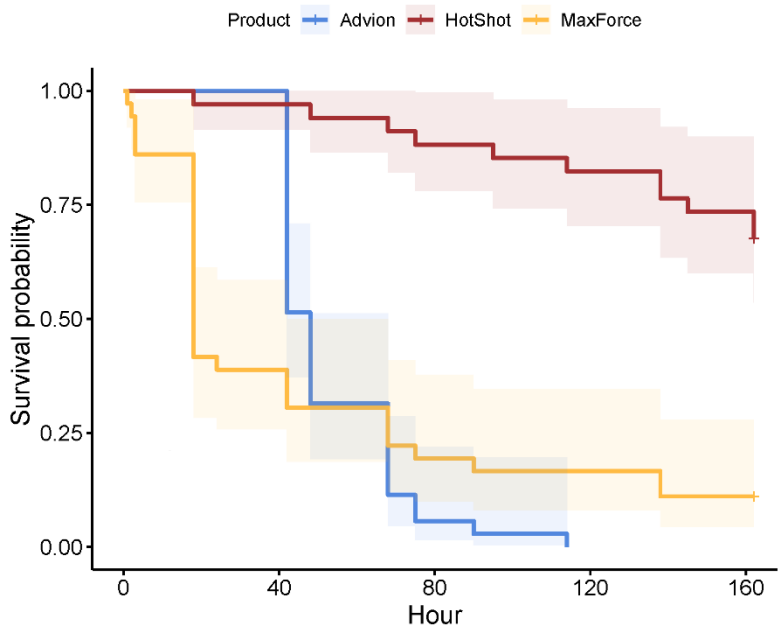

Figure 5.7. Survivorship trends for *P. americana* exposed to dried applications of Advion, Hot Shot, and Maxforce cockroach insecticidal gel treatments.

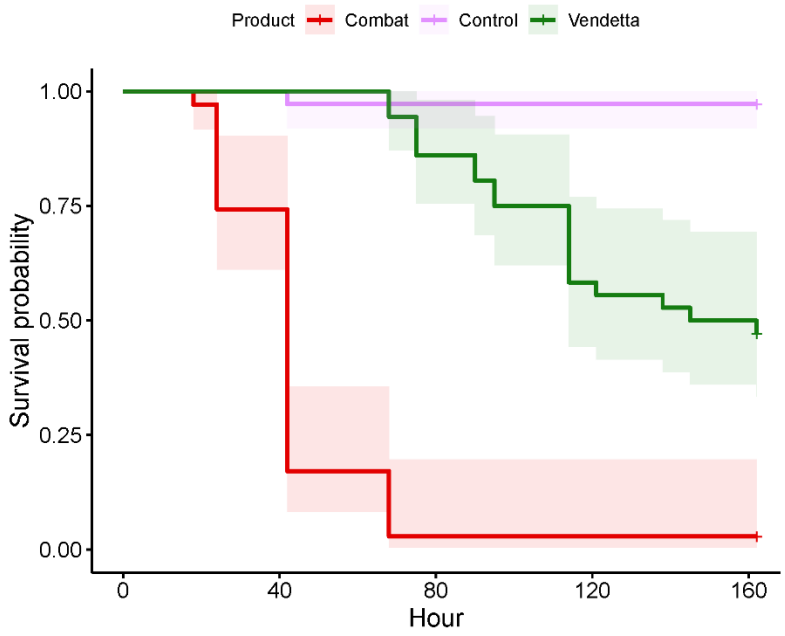

Figure 5.8. Survivorship trends for *P. americana* exposed to dried applications of Combat and Vendetta cockroach insecticidal gel treatments as well as the control (empty weigh boat).

Table 5.1. Manufacturer information and gel characteristics for each bait formulation used in this study. The proportion of active ingredient is based on each manufacturer's listing. The total proportion of water was determined by weighing oven dried gel samples, and proportion of water lost at 24 h been calculated from weighing of gel samples after 24 h of air drying.

Brand	Manufacturer	Active Ingredient	% Active Ingredient	% Water Total (SEM)	% Water Lost at 24 h (SEM)	Gel Characteristics	EPA Reg. No.
Advion	Syngenta Group	Indoxacarb	0.60	70.73 (0.70)	59.15 (0.51)	Tan; Sour Scent	100-1484
Maxforce Impact	Bayer	Clothianidin	1.00	60.35 (0.56)	51.24 (0.47)	Tan; Lightly Sour Scent; Oily	432-1531
Combat Max	Henkel	Fipronil	0.01	48.36 (0.76)	38.83 (0.84)	Brown; Yeast Scent	64240-45
Hot Shot	United Industries	Dinotefuran	0.05	91.34 (1.33)	86.88 (1.02)	Clear; Slightly Sweet Scent	9688-271-8845
Vendetta	MGK	Abamectin B1	0.05	56.92 (0.87)	48.28 (0.95)	Light Brown; Strong Yeast Scent	1021-1828

Table 5.2. Median survival time, LT_{50}, and percent final mortality data for freshly applied gel formulations tested on *A. hagenowii*. The test statistic and P-value for comparisons of median survival and final mortality with their respective controls is provided in parenthesis. The Bonferroni method was used for analysis of survival curve, and two-tailed Z-test analysis was used for final mortality comparisons. Survival and mortality comparisons in bold are significantly different at $P \leq 0.05$, with LT_{50} comparisons considered significant if 95% CI does not overlap with the control CI.

Treatment	n	Median Survival (h)	Survival Curve Comparison	LT_{50} (h)	Final Mortality (%)
Advion	24	168 (CI = 81.89-254.12)	0.249, *P* = 1.00	171.20 (CI = 146.44-214.71)	58.33 (0.596, *P* = 0.548)
Maxforce Impact	24	24 (CI = 10.80-37.20)	**32.342, *P* < 0.001**	**19.99 (CI = 14.91-25.79)**	**95.83 (2.589, *P* < 0.01)**
Combat Max	24	48 (CI = 43.45-52.55)	**37.448, *P* < 0.001**	**28.22 (CI = 23.23-32.85)**	**100.00 (3.098, *P* < 0.01)**
Hot Shot	24	24 (CI = 14.01-34.00)	**35.707, *P* < 0.001**	**11.72 (CI = 8.20-16.77)**	87.50 (1.717, *P* = 0.085)
Vendetta	24	120 (CI = 62.40-177.61)	5.858, *P* = 0.233	**113.83 (CI = 91.33-148.27)**	75.00 (0.6351, *P* = 0.522)
Control	24	192 (CI = 163.29-220.70)	-	200.64 (CI = 170.49-262.52)	66.67

Table 5.3. Median survival time, LT_{50}, and percent final mortality data for dried gel formulations tested on *A. hagenowii*. The test statistic and P-value for comparisons of median survival and final mortality with their respective controls is provided in parenthesis. The Bonferroni method was used for analysis of survival curve, and two-tailed Z-test analysis was used for final mortality comparisons. Survival and mortality comparisons in bold are significantly different at $P \leq 0.05$, with LT_{50} comparisons considered significant if 95% CI does not overlap with the control CI.

Treatment	n	Median Survival (h)	Survival Curve Comparison	LT_{50} (h)	Final Mortality (%)
Advion	24	72 (CI = 56.64-87.36)	2.815, *P* = 1.00	63.07 (CI = 54.56-71.60)	95.83 (1.139, *P* = 0.254)
Maxforce Impact	24	24 (CI = 16.84-31.16)	**36.278, *P* < 0.001**	**3.53 (CI = 2.38-5.05)**	100.00 (1.871, *P* = 0.061)
Combat Max	24	48 (CI = 43.10-52.90)	**30.095, *P* < 0.001**	**27.29 (CI = 21.98-31.94)**	100.00 (1.871, *P* = 0.061)
Hot Shot	24	96 (CI = 82.79-109.21)	2.347, *P* = 1.00	66.19 (CI = 57.71-74.38)	95.83 (1.139, *P* = 0.254)
Vendetta	24	24 (CI = 10.49-37.51)	**15.826, *P* < 0.01**	**22.88 (CI = 14.15-37.74)**	79.17 (0.643, *P* = 0.522)
Control	22	96 (CI = 74.03-117.97)	-	81.55 (CI = 71.19-93.91)	86.36

Table 5.4. Pairwise comparisons (Bonferroni method) of survival curve between fresh gel formulations tested on *A. hagenowii*. Each treatment has N = 24, and comparisons in bold are significantly different at $P \leq 0.05$.

Treatment	Advion	Maxforce Impact	Combat Max	Hot Shot	Vendetta
Advion	-	**32.039, *P* < 0.001**	**39.036, *P* < 0.001**	**32.262, *P* < 0.001**	2.491, *P* = 1.00
Maxforce Impact	**32.039, *P* < 0.001**	-	0.441, *P* = 1.00	1.560, *P* = 1.00	**17.183, *P* < 0.001**
Combat Max	**39.036, *P* < 0.001**	0.441, *P* = 1.00	-	3.528, *P* = 0.905	**18.695, *P* < 0.001**
Hot Shot	**32.262, *P* < 0.001**	1.560, *P* = 1.00	3.528, *P* = 0.905	-	**20.290, *P* < 0.001**
Vendetta	2.491, *P* = 1.00	**17.183, *P* < 0.001**	**18.695, *P* < 0.001**	**20.290, *P* < 0.001**	-

Table 5.5. Pairwise comparisons (Bonferroni method) of survival curve among dried gel formulations tested on *A. hagenowii*. Comparisons in bold are significantly different at $P \leq 0.05$.

Treatment	Advion	Maxforce Impact	Combat Max	Hot Shot	Vendetta
Advion	-	**34.865, *P* < 0.001**	**23.622, *P* < 0.001**	0.051, *P* = 1.00	**12.301, *P* < 0.01**
Maxforce Impact	**34.865, *P* < 0.001**	-	**15.535, *P* < 0.01**	**36.395, *P* < 0.001**	**13.715, *P* < 0.01**
Combat Max	**23.622, *P* < 0.001**	**15.535, *P* < 0.01**	-	**25.047, *P* < 0.001**	0.001, *P* = 1.00
Hot Shot	0.051, *P* = 1.00	**36.395, *P* < 0.001**	**25.047, *P* < 0.001**	-	**13.876, *P* < 0.01**
Vendetta	**12.301, *P* < 0.01**	**13.715, *P* < 0.01**	0.001, *P* = 1.00	**13.876, *P* < 0.01**	-

Table 5.6. Median survival time, LT_{50}, and percent final mortality data for freshly applied gel formulations tested on P. *americana*. The test statistic and *P*-value for comparisons of median survival and final mortality with their respective controls is provided in parenthesis. The Bonferroni method was used for analysis of survival curve, and two-tailed Z-test analysis was used for final mortality comparisons. Survival and mortality comparisons in bold are significantly different at $P \leq 0.05$, with LT_{50} comparisons considered significant if 95% CI does not overlap with the control CI.

Treatment	n	Median Survival (h)	Survival Curve Comparison	LT_{50} (h)	Final Mortality (%)
Advion	36	40 (CI = 34.12-45.88)	**64.074, *P* < 0.001**	**41.93 (CI = 32.25-50.91)**	**97.22% (7.932, *P* < 0.001)**
Maxforce Impact	35	65 (CI = 40.24-89.76)	**60.637, *P* < 0.001**	**45.63 (CI = 29.20-66.59)**	**94.29% (8.192, *P* < 0.001)**
Combat Max	36	40 (CI = 36.41-43.59)	**66.844, *P* < 0.001**	**29.34 (CI = 17.15-39.59)**	**100.00% (8.485, *P* < 0.001)**
Hot Shot	35	40 (CI = 0.00-95.63)	**46.197, *P* < 0.001**	**25.86 (CI = 16.91-38.14)**	**80.00% (6.694, *P* < 0.001)**
Vendetta	38	118 (CI = 93.84-142.16)	**40.606, *P* < 0.001**	**108.47 (CI = 97.27-123.57)**	**73.68% (6.532, *P* < 0.001)**
Control	36	165	-	165	0.00

Table 5.7. Median survival time, LT_{50}, and percent final mortality data for dried gel formulations tested on P. *americana*. The test statistic and *P*-value for comparisons of median survival and final mortality with their respective controls is provided in parenthesis. The Bonferroni method was used for analysis of survival curve, and two-tailed Z-test analysis was used for final mortality comparisons. Survival and mortality comparisons in bold are significantly different at $P \leq 0.05$, with LT_{50} comparisons considered significant if 95% CI does not overlap with the control CI.

Treatment	n	Median Survival (h)	Survival Curve Comparison	LT_{50} (h)	Final Mortality (%)
Advion	35	48 (CI = 37.23-58.77)	**61.247, P < 0.001**	**42.74 (CI = 39.08-46.16)**	**100.00% (8.192, P< 0.001)**
Maxforce Impact	36	18 (CI = 12.56-23.43)	**52.446, P < 0.001**	**17.95 (CI = 13.78-22.47)**	**88.89% (7.332, P < 0.001)**
Combat Max	35	42 (CI = 38.07-45.93)	**58.775, P < 0.001**	**32.64 (CI = 25.69-38.99)**	**97.14% (7.951, P < 0.001)**
Hot Shot	34	*144 (130.67-156.92)	**10.053, P = 0.023**	**401.94 (CI = 250.93-1143.56)**	**32.24% (3.281, P = 0.001)**
Vendetta	35	†145	**20.484, P < 0.001**	**137.73 (CI = 127.16-153.21)**	**54.29% (4.824, P < 0.001)**
Control	36	*159	-	-	2.78%

* Unable to calculate median survival time and CI. †Unable to calculate CI. When available, mean survival time and CI is provided as an approximation of median survival time.

Table 5.8. Pairwise comparisons (Bonferroni method) of survival curves among fresh gel formulations tested on *P. americana*. Comparisons in bold are significantly different at $P \leq 0.05$.

Treatment	Advion	Maxforce Impact	Combat Max	Hot Shot	Vendetta
Advion	-	4.177, P = 0.615	**18.441, P < 0.001**	0.160, P = 1.000	**34.688, P < 0.001**
Maxforce Impact	4.177, P = 0.615	-	**12.300, P < 0.01**	0.779, P = 1.00	**12.577, P < 0.01**
Combat Max	**18.441, P < 0.001**	**12.300, P < 0.01**	-	0.009, P = 1.00	**53.403, P < 0.001**
Hot Shot	0.160, P = 1.00	0.779, P = 1.00	0.009, P = 1.00	-	**9.454, P = 0.032**
Vendetta	**34.688, P < 0.001**	**12.577, P < 0.01**	**53.403, P < 0.001**	**9.454, P = 0.032**	-

Table 5.9. Pairwise comparisons (Bonferroni method) of survival curves among dried gel formulations tested on *P. americana*. Comparisons in bold are significantly different at $P \leq 0.05$.

Treatment	Advion	Maxforce Impact	Combat Max	Hot Shot	Vendetta
Advion	-	**8.794, *P* = 0.045**	**12.126, *P* = 0.007**	**49.359, *P* < 0.001**	**58.445, *P* < 0.001**
Maxforce Impact	**8.794, *P* = 0.045**	-	5.305, *P* = 0.319	**37.524, *P* < 0.001**	**35.795, *P* < 0.001**
Combat Max	**12.126, *P* = 0.007**	5.305, *P* = 0.319	-	**48.455, *P* < 0.001**	**57.907, *P* < 0.001**
Hot Shot	**49.359, *P* < 0.001**	**37.524, *P* < 0.001**	**48.455, *P* < 0.001**	-	2.993, *P* = 1.000
Vendetta	**58.445, *P* < 0.001**	**35.795, *P* < 0.001**	**57.907, *P* < 0.001**	2.993, *P* = 1.000	-