



A female sterilization method for use in field-based behavioral studies of the invasive Asian longhorned beetle (*Anoplophora glabripennis*)

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Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky), a wood borer (Coleoptera: Cerambycidae) native to China, has been unintentionally and repeatedly introduced to North American and European landscapes as a stow-away in the wood packing material commonly used in international trade. Asian longhorned beetle causes extensive damage and mortality in multiple deciduous tree species and in response, countries in both North America and Europe have adopted policies of eradication. Models that integrate patterns of Asian longhorned beetle dispersal with records of infested trees are critical in optimizing survey and eradication efforts and tracking eradication progress. While these tools continue to be developed, they have been limited by the availability of experimental dispersal data. Existing data is restricted to observations made in the beetle's native range in China or based on inference of dispersal in invaded landscapes. Direct observation of beetle dispersal behavior in invaded landscapes could provide critical behavioral information, but the experimental release of gravid females has been incompatible with eradication program efforts. To fill this knowledge gap, there is a need to identify field-portable methods of effectively sterilizing mated females that do not alter ovipositional behavior. Here, we present a protocol for cauterizing a beetle's ovipositor to prevent successful oviposition. Results of lab trials demonstrate the efficacy of ovipositor cauterization in inhibiting successful oviposition without altering the egg-laying behavior of gravid Asian longhorned beetle females. This method enables research to inform models of beetle dispersal and infestation risk without adding to actual or perceived risk of exacerbating infestations in an eradication program.

Key words: cauterization, eradication, exotic, pest management, wood borer

Introduction

The invasive Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky) has potential to be one of the most damaging wood borers (Coleoptera: Cerambycidae) in Europe and North America due to the ability of this globally invasive species to feed on a broad range of host tree species spread across more than 15 families (Hérard et al. 2006, Haack et al. 2010, Wang 2015). Asian longhorned beetle has been inadvertently introduced to North American and European landscapes through the movement of infested wood packaging material (e.g., crates and pallets) commonly used in international shipping (Haack et al. 2010). Invasive, localized populations of Asian longhorned beetle have been found in the United States, Canada, France, Germany, Italy, Belgium, the Netherlands, Switzerland, the United Kingdom, Finland, and Montenegro (Javal et al. 2019, Javal 2020). In the United States alone, Asian longhorned beetle has the potential to kill up to 30% of urban trees (1.2 billion trees) and reduce urban canopy cover by 35% at an estimated cost of \$669 billion

in 2001 (Nowak et al. 2001), or over \$1.2 trillion when adjusted for inflation (<https://data.bls.gov/cgi-bin/cpicalc.pl>, accessed 26 August 2024).

The methods used to eradicate invasive populations of Asian longhorned beetle in North America have focused on using surveys to identify infested trees. The distribution of these trees is then used to establish a quarantine zone from which the movement of potentially infested material is regulated and within which infested trees are removed and destroyed (Haack et al. 1997, Rose 2014, Turgeon et al. 2022). At its core, this approach depends on visually surveying each potential host tree on the landscape, searching the trees for exit holes and oviposition pits either from the ground by personnel using binoculars, from elevated positions using bucket trucks, or from within the canopy by trained tree climbers. Because visual surveys are not 100% effective at detecting infestations, stands may require multiple surveys to achieve an acceptable reduction in risk. On landscapes that can span more than 250 km² that include millions of

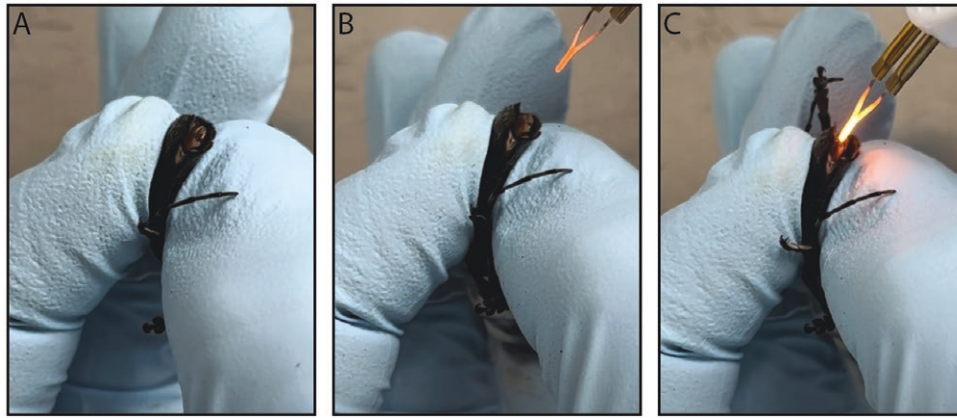


Fig. 1. A) During the cauterization sterilization procedure the beetle can be restrained with a microcentrifuge tube or towel to prevent movement and bites. B) The ovipositor is everted by putting gentle inward and downward force on the last abdominal sternite with the thumb. C) Then the posterior end of the ovipositor is cauterized with a battery-powered cautery pen to prevent the ovipositor from everting fully to deposit eggs under bark..

host trees across complex landscapes, these surveys are both costly and time-consuming. The scope of the challenge requires substantial investment from and coordination between federal (U.S. Animal and Plant Health Inspection Service, U.S. Forest Service), state, and local agencies (Haack et al. 1997, Rose 2014). Eradication programs in the United States have cost an estimated \$249 million between 1998 and 2006 (Smith et al. 2009). With eradication efforts ongoing in Ohio, Massachusetts, New York, and South Carolina, the cost of Asian longhorned beetle eradication in the United States continues to rise.

Because surveys make up the largest portion of eradication costs, recent work has focused on modeling Asian longhorned beetle dispersal and risk of infestation across quarantined landscapes (Trotter and Hull-Sanders 2015, Kappel et al. 2017, Trotter et al. 2019, 2021, 2023) to help prioritize and inform survey strategies and to quantify progress toward eradication. By integrating results of tree surveys and records of host removals, Trotter et al. (2023) have modeled the reduction in Asian longhorned beetle risk due to actions taken by the Cooperative Asian Longhorned Beetle Eradication program managed by the U.S. Animal and Plant Health Inspection Service, and state and local eradication partners. This Asian longhorned beetle risk model may also be used to inform eradication program planning of where to survey, optimal periods for resurvey, and the extent of regulated areas (Trotter et al. 2023). While multiple studies of Asian longhorned beetle movement have been conducted in China (Zhou et al. 1984, Smith et al. 2001, 2004, Williams et al. 2004, Bancroft and Smith 2005), improving model accuracy requires further study of Asian longhorned beetle behavior in its North American invasive range, specifically at low densities and in urban landscapes. Such studies will improve our ability to accurately model how Asian longhorned beetle adults select hosts for oviposition and feeding on landscapes with variable host availability and how dispersing females disperse on heterogeneous landscapes that include wooded areas, stand edges, and open spaces including bodies of water and impervious surfaces, features common on an urban landscape.

Since populations of Asian longhorned beetle outside of its native range are under management for eradication, the release of live marked individuals in the field, even in locations with active infestations, includes some risk of exacerbating the infestation. The escape of gravid females has potential to lead to the infestation of additional host trees, and/or introduction of new genetic variation (depending on the source of study individuals). This is of particular

concern if studies are to be conducted in areas where the species is only present in quarantined infestations. Further, the release of gravid females creates an opportunity for negative public perception of eradication programs. To help address these risks while supporting the study of dispersal in invaded landscapes, we developed a method of preventing oviposition by gravid females.

Experimental Design

We explored multiple methods of preventing oviposition by gravid Asian longhorned beetle females including the use of cyanoacrylate adhesives (Super Glue) and multiple methods of cauterization. Of these, only the method described below was effective and consistent at preventing gravid Asian longhorned beetle females from placing eggs under bark without altering oviposition behavior. During and after mating, female Asian longhorned beetle chew round oviposition pits in the bark of their preferred host. When the bark of the host is thin, the oviposition site may consist of only a thin slit while on hosts with thicker bark the pits may be oblong and up to 1 cm in width (Keena and Sánchez 2018). After chewing a suitable oviposition pit, the female turns 180° and inserts its ovipositor under the bark, prying the bark up to create a cavity into which it deposits a single egg (Keena and Sánchez 2018). Under laboratory conditions a single female may deposit as many as 170 eggs with 92% viability (Keena 2002).

We have found that using a battery-powered electric cautery pen to cauterize the posterior end of the ovipositor (see Procedure below; Figs. 1 and 2) prevents a female beetle from fully extending its ovipositor, making it impossible for the female to deposit eggs under bark. We tested the efficacy of this method under quarantine conditions using adults from lab-reared colonies. Additional information on the methods used to maintain the colony of beetles used in this study is detailed by Keena (2005) and Keena and Sánchez (2018).

To test the efficacy of this cauterization sterilization method we set up 52 adult mating pairs, each in its own 3.8-liter (1-gallon) jar containing fresh maple (*Acer platanoides*) twigs for adults to feed upon and a single maple bolt ~20 cm in length, and between 3 and 8 cm in diameter (Keena 2005). The ends of the freshly cut bolts were coated in melted paraffin wax to help maintain the moisture content of the wood. Males and females were placed together in the jars and were given 2 days to mate. After this period the males were removed, and the females were given an additional 2 days to

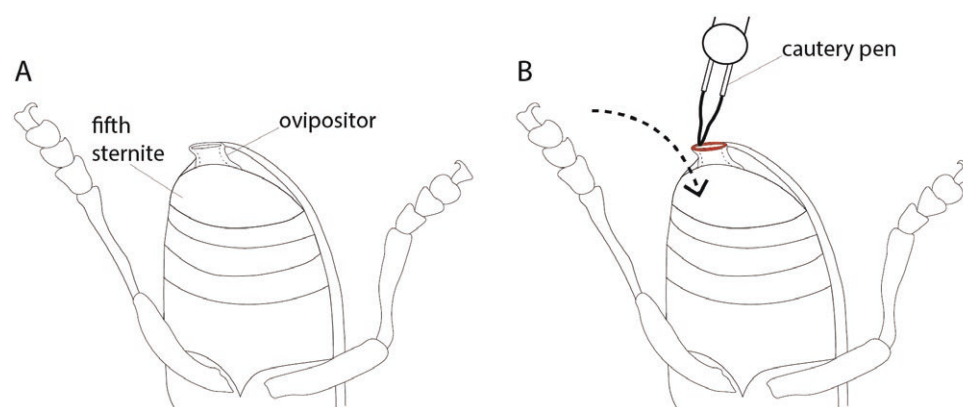


Fig. 2. A) The anatomy of the posterior end of an adult female Asian longhorned beetle (*Anoplophora glabripennis* Motschulsky). B) To evert the ovipositor, pressure is applied to the last abdominal sternite in an inward and downward motion (dashed arrow), then the distal end of the ovipositor (highlighted) is cauterized with a cautery pen.

oviposit prior to treatment. Bolts exposed to females prior to treatment were removed from mating jars and held in a growth chamber set at 25°C (16:8 L:D) to allow eggs to develop, and after a minimum of 7 days, were peeled to confirm females were gravid. The number of oviposition pits chewed per bolt was tracked for a subset of pretreatment females. Immediately after removing pretreatment bolts, half of females were selected at random, and the ovipositors were cauterized to prevent oviposition. The remaining females were left intact as a control treatment and did not undergo the additional handling necessary for cauterization. All females (treatment and control) were then placed in individual jars with a fresh bolt and fresh twigs. Beetles were held on the bolts for another 7 days to allow for oviposition. After the 7-day trial, bolts were removed and placed in a growth chamber set at 25°C where they were held for a minimum of 7 more days. After this holding period, we recorded the number of oviposition pits in the bark, then peeled the bark to determine whether the pits contained viable Asian longhorned beetle eggs or larvae (Keena 2005). Due to constraints on the availability of live beetles and the availability of growth chamber space, the beetles were processed as 3 separate sequential groups. Observations from females that died during trials, failed to oviposit pre-cauterization, or from trials in which the ovipositor could not be exposed and visualized during cauterization were dropped from analyses, though we did peel bolts from these trials to observe the number of successfully oviposited eggs.

All analyses were conducted in program R version 4.4.0 (R Core Team 2024). We analyzed the effect of cauterization treatment on rates of oviposition pit chewing and egg laying by fitting 2 separate linear mixed models with package “lme4” (Bates et al. 2015) with fixed effects for treatment, period (i.e., before or after treatment), the interaction of treatment and period, and a random effect for individual female identification. Post hoc tests were conducted on significant interaction terms using the “emmeans” package (Lenth 2020).

Procedure

1) *Preparation and Handling*—Assemble the battery-powered electric cautery pen (Bovie Reusable Change-A-Tip Deluxe Cautery Kit, Bovie Medical Corporation/Apyx Medical, Clearwater, Florida) and insert one of the fine tips (item H101) included in the kit. During the procedure, the beetle can be restrained with a microcentrifuge tube (1.5 ml or 5.0 ml cut to different lengths to accommodate different beetle sizes) or a folded paper

towel to prevent movement and avoid bites (note that the beetle is capable of delivering a substantial bite with its mandibles). If a microcentrifuge tube is used to restrain the beetles during cauterization, make sure that the tube is large enough for each beetle, as beetles can be hard to remove from the tube if it is not properly sized. After placing the head of the beetle in the tube or towel, the handler holds the beetle with the head down and ventral side facing them. The ovipositor is exposed and under optimal conditions, is partially everted by putting gentle inward and downward force on the last abdominal (fifth) sternite with the thumb (Fig. 1A). If the ovipositor is not fully visible during handling, do not attempt cauterization. In trials, some attempts to cauterize ovipositors that were not visible resulted in beetle death due to internal damage or beetles capable of laying eggs after cautery treatment. However, if cautery is performed correctly, beetles can survive weeks after cautery without successfully depositing eggs (J.L.C., pers. obs.).

- 2) *Cauterization*—With the female restrained and the ovipositor exposed or partially everted, use the free hand to pick up the cautery pen. Press the button on the pen to allow it to heat fully just prior to touching the beetle. Using quick (<1 s), gentle taps with the tip of the heated cautery pen, burn the entire circumference of the distal end of the ovipositor (Figs. 1 and 2; Supplementary Video 1). Burning the ovipositor will prevent it from fully everting and prevents females from successfully ovipositing an egg under bark. Recent studies have presented evidence for pain and/or nociception in insects (e.g., Adamo 2019, Gibbons et al. 2022, Crump et al. 2023), which suggests that this procedure may induce pain in beetles. Researchers should consider how pain could alter beetle behavior and may want to take measures to mitigate the perception or effects of pain.
- 3) *Post-cautery observation*—Before marking and releasing individuals for behavioral studies, we recommend holding cauterized females for 24 h prior to release to ensure that the cauterization treatment did not injure the female in a way that would affect behavior.

Results

Overall, the cauterization treatment method was successful in preventing under-bark oviposition by 95% of the treated females. Five females that died during the posttreatment portion of trials (2 in the control group and 3 in the cautery treatment group) were

excluded from analyses yielding a sample size of 24 females in the control group and 20 females in the cautery group. We also excluded observations from 2 trials in which the ovipositor could not be exposed and visualized; these were females which would not have been released in a field setting, a precaution which proved conservative yet warranted as one of these 2 females went on to successfully oviposit.

Across both treatment groups, 430 eggs were oviposited in the 4 days pretreatment and 464 eggs were oviposited in the 7 days posttreatment. Only 19 eggs (4.09%) of posttreatment eggs were in cauterization treatment trials; all these eggs were laid by a single female, representing a treatment failure rate of 5.00% (1 of 20 cauterized females). This cauterization failure was due to the inability of the handler to fully expose the ovipositor during treatment and is the rationale for making the exposure of the ovipositor a requirement for successful sterilization. Females should not be released in the field unless the handler is able to completely expose the ovipositor by applying pressure to the last abdominal sternite.

A total of 4 cauterized females were able to pass an egg through their ovipositor; however, without intact ovipositors, the females were unable to place eggs under bark: one of these females was found with an egg stuck to the posterior tip of its abdomen, and the remaining 3 females laid eggs that were found on the bottom of the mating jar or on the surface of the bark. Since eggs oviposited outside of a host under field conditions would be exposed to elevated risk of desiccation, predation, and fungal attack, they are unlikely to develop, hatch, and chew into the tree. Therefore, eggs that were not oviposited under bark were considered nonviable.

The rate of oviposition (number of eggs laid per day) was significantly influenced by the interaction of treatment and period ($X^2 = 43.205$, $df = 1$, $P < 0.001$) and the main effect of period ($X^2 = 67.004$, $df = 1$, $P < 0.001$) but not treatment ($X^2 = 0.001$, $df = 1$, $P = 0.973$). Rate of oviposition decreased after treatment for cauterized females (difference = 2.302 ± 0.281 eggs per day, $t = 8.186$, $df = 42$, $P < 0.001$) but did not change for control females ($t = -0.783$, $df = 42$, $P = 0.438$; Fig. 3A). The rate of eggs laid did not differ between treatment groups prior to cauterization ($t = -0.034$, $df = 79.4$, $P = 0.973$). Despite the significant effect of treatment on successful oviposition, the rate of chewing oviposition pits (pits per day) was not significantly influenced by treatment group ($X^2 = 0.139$, $df = 1$, $P = 0.710$), period ($X^2 = 0.832$, $df = 1$, $P = 0.362$), nor their interaction ($X^2 = 2.300$, $df = 1$, $P = 0.129$; Fig. 3B) suggesting the rate of attempted oviposition is constant between the groups.

Discussion

The Asian longhorned beetle ovipositor cauterization protocol provides a novel method of preventing oviposition by gravid Asian longhorned beetle females. Dispersal of Asian longhorned beetle has yet to have been directly studied in its invasive habitat in North America due to concerns that study beetles could escape and infest new trees. Therefore, models of Asian longhorned beetle dispersal in North America have thus far been parameterized using data from lab studies, research conducted in the beetle's native range, or eradication program surveys. This beetle sterilization technique would allow for the first direct observation of dispersal, oviposition, and host selection behavior of gravid females in an invasive Asian longhorned beetle population with a significantly reduced risk of creating additional infestations.

Results from our observations during lab trials of this method indicate that cauterization does not significantly impact typical chewing and oviposition behavior by gravid females. Therefore, this protocol enables the use of any standard method of marking and field-tracking individual beetles (e.g., fluorescent marking and following, mark-recapture, harmonic radar) to record observations of host selection and dispersal behavior in locations where the species is invasive/regulated with reduced risk of introducing new individuals or genetics into an invasive population. Due to the high risk of accidental introduction of Asian longhorned beetle in shipping materials worldwide (Javal et al. 2019), this method has potential for high impact and widespread application.

It must be noted that the use of the cauterization sterilization method does not completely eliminate the possibility of oviposition. In our evaluation of this method, 1 female was still able to oviposit under bark after attempted cauterization. The failure to prevent oviposition with this individual highlights the risk of cauterization failure when ovipositor eversion is not successfully, and risk can be minimized by not releasing females which were difficult to cauterize; cauterization can fail if the handler is unable to get the female to expose or partially evert its ovipositor and cannot visualize the area to be cauterized. Additionally, 4 females were able to pass eggs through their ovipositor, but not oviposit under bark. Whereas there is minimal risk that an egg on the surface of a tree would survive desiccation and predation and yield a larva that is able to chew its way into a host, we have no empirical estimate of eggs laid outside of a host in the field. While alternatives to studying Asian longhorned beetle in its invasive range (i.e., under quarantine in a lab setting or in Asian

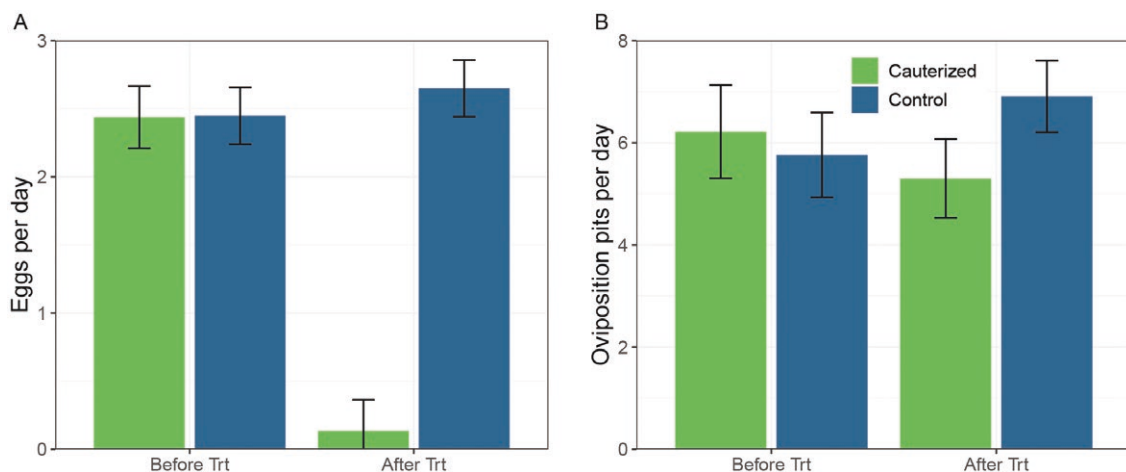


Fig. 3. The rates of A) oviposition (eggs laid per day) and B) oviposition pit chewing (pits per day) before and after treatment for each group: a group that received ovipositor cauterization to prevent the oviposition of eggs under bark and a no-treatment control group.

longhorned beetle's native range), may present no risk, foreign travel and establishing or maintaining quarantine facilities is often prohibitively expensive or logistically infeasible. Furthermore, the minimal risk inherent in releasing cauterized Asian longhorned beetle females may be outweighed by the benefits of increased management accuracy and efficiency gained from parameterizing invasive Asian longhorned beetle dispersal models using behavioral data collected in its invasive habitat.

Supplementary data

Supplementary data are available at *Journal of Insect Science* online.

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Author contributions

Jennifer Chandler (Conceptualization [equal], Data curation [lead], Formal analysis [lead], Investigation [lead], Methodology [equal], Visualization [lead], Writing—original draft [lead], Writing—review & editing [equal]), and Talbot Trotter (Conceptualization [equal], Funding acquisition [lead], Investigation [supporting], Methodology [equal], Project administration [lead], Resources [lead], Writing—review & editing [equal])

Data availability

Data supporting the conclusions of this study are available in [Supplementary Table S1](#).

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