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The Lari-Leuco Container: A Novel Collection Arena for Separating Insects Ascending or Descending From a Plant Foliage Sample

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Abstract

Efficient separation of insects from plant material for quantification and collection is an important component of entomological research. This paper reports on a novel, easily replicable container designed to efficiently collect two different biological control agents dispersing from hemlock (*Tsuga* spp.) foliage infested with the invasive hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae). The container utilizes a simplified Berlese-style funnel design to collect *Laricobius* spp. (Coleoptera: Derodontidae) larvae dropping from the foliage into a removable bottom jar, a central jar to house the foliage sample, and a removable top jar to collect adult silver flies (*Leucopis* spp., Diptera: Chamaemyiidae) emerging from puparia on the twigs. The efficacy of two designs (with and without a funnel leading to the top collection jar) was evaluated using western hemlock [*Tsuga heterophylla* (Raf.) Sarg.] foliage naturally colonized with HWA and the two predator genera. All *Laricobius* larvae were effectively collected in the bottom jar, and the addition of an inverted funnel leading to the top collection jar increased the proportion of *Leucopis* flies reaching the target jar from 60% to 94%. This 'Lari-Leuco' container is presented as a research and motoring tool to benefit the integrated pest management program for HWA in eastern North America and for potential use in simultaneously separating ascending and descending life stages in other insect-plant or predator-prey systems.

Keywords: insect collection, insect rearing, silver flies, hemlock woolly adelgid, biological control

Among the indispensable tools in entomological research are containers for rearing and observing live insects. Such containers are commonly customized to suit the type of material collected, the behavioral, environmental, nutritional requirements of the insects, and the specific needs of the investigator (Cothran and Gyrisco 1966, Singh and Surrey 1980, Cohen 2018). Containers that separate insects from their host plants or other natural materials using gravity, light, temperature, or other environmental gradients are particularly useful for quantifying and collecting certain species (Browne 1972, Schauff 2001, Keena 2017), including those used in biological control (Elkinton et al. 2021, Foley et al. 2021).

The hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Hemiptera: Adelgidae) is a destructive, invasive forest pest in eastern North America on eastern hemlock (*Tsuga canadensis* (L.) Carrière) and Carolina hemlock (*Tsuga caroliniana* Engelmann) (Havill et al. 2016). Sessile adults of the sistens generation produce woolly ovisacs on host twigs in late winter/early spring and give rise to a progrediens generation in late spring/early summer (McClure 1989, Joseph et al. 2011). The biological control program for HWA (Onken and Reardon 2011) has emphasized the use of two predators in the genus *Laricobius* (Coleoptera: Derodontidae). *Laricobius nigrinus* Fender is native to the Pacific Northwest region of North America and has been established in the eastern United States since releases began in 2003 (Mausel et al. 2010). Adult *La. nigrinus* are active in the winter and lay their eggs in HWA sistens ovisacs, where larvae feed on HWA eggs before dropping from branches in the spring to pupate in soil (Zilahi-Balogh et al. 2003). Its congener, *Laricobius osakensis* Montgomery and Shiyake, is native to Japan, has been released in the United States since 2012, has become established at several sites, and has a life cycle similar to *La. nigrinus* (Vieira et al. 2013, Toland et al. 2018).

More recently, western North American lineages of two silver fly species, *Leucopis argenticollis* Zetterstedt and *Leucopis piniperda* Malloch, are being evaluated as additional biological control agents for HWA in eastern North America (Kohler et al. 2008, Havill et al. 2018). Collectively, these two *Leucopis* species prey on both HWA generations, are commonly associated with *La. nigrinus* on western hemlock, *Tsuga heterophylla* (Raf.) Sarg. (Kohler et al. 2016, Rose et al. 2020), and complete development in the field on the HWA lineage that feeds on eastern hemlock (Motley et al. 2017). Unlike *Laricobius*, these *Leucopis* species pupate on the twigs after the larvae feed on HWA eggs and disperse from the foliage as winged adults (Grubin et al. 2011). Releases of *Le. argenticollis* and *Le. piniperda* have been made in the eastern United States since 2015 and efforts to monitor for their establishment are ongoing (New York State Hemlock Initiative 2021). Thus, there is an interest by researchers and managers to monitor for the field establishment of both *Laricobius* and *Leucopis* in areas where predators in both genera are being released.

To aid these research and monitoring efforts, we hereby introduce and evaluate a container designed to simultaneously separate *Laricobius* larvae and *Leucopis* adults as they descend and ascend, respectively, from HWA-infested hemlock foliage. This 'Lari-Leuco' (*Laricobius* + *Leucopis*) container isolates the target predator groups into separate removable jars where they can be easily collected and quantified as live specimens. Because *Leucopis* is not yet known to be established on hemlock in eastern North America, we used western hemlock foliage colonized by HWA, *La. nigrinus*, *Le. argenticollis*, and *Le. piniperda* in our evaluations. The advantages of the Lari-Leuco container relative to other collection arenas for HWA predators are discussed.

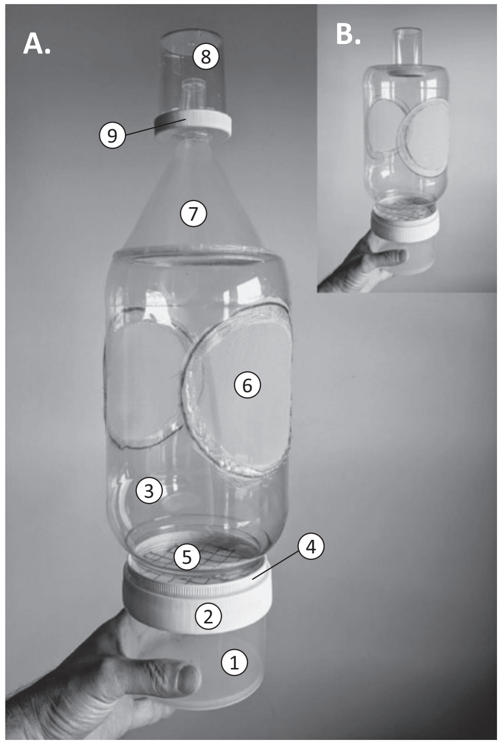

Fig. 1. (**A**) Components of the Lari-Leuco container: 1) a 500-ml round, straight-sided plastic jar, 2) screw cap to a 500-ml jar, 3) a 3.8-liter round plastic jar, 4) screw cap to a 3.8-liter jar, 5) a 10.5 cm diameter round piece of steel hardware cloth (1.27 cm opening), 6) a 12.5-cm diameter round piece of polyester mesh (435 µm opening), 7) a 12-cm diameter round plastic funnel, 8) a 118-ml round straight-sided plastic jar, 9) screw cap to a 118-ml jar. (**B**) Preliminary version of the container without the plastic funnel.

Materials and Methods

Constructing the Container

The Lari-Leuco container was constructed using nine components (Fig. 1A) plus hot glue adhesive. **Base:** The bottom jar was a 500-ml clear, round, wide-mouth, straight-sided, plastic (PPCO) Nalgene jar with a plastic (PP) screw cap enclosure with recessed top (product #2118-0016, Thermo Fisher Scientific, Waltham, MA, USA). A 10-cm diameter hole was cut in the center of the screw cap using a rotary saw (all holes were cut by first anchoring an expendable jar/cap to an immovable surface using clamps or screws and firmly attaching the paired cap/jar to be cut). A 10-cm hole was also cut in the center of the plastic (PP) screw cap of a 3.8-liter clear, round, wide-mouth, plastic (PET) jar (product S-18077, Uline, Pleasant Prairie, WI, USA). The screw cap of the 3.8-liter jar was then adhered top-side-down to the top of the screw cap of the 500-ml jar using a hot glue gun (Arrow Fastener Co., Saddle Brook, NJ, USA), with the 10-cm holes in each cap aligned. A circular piece of galvanized steel hardware cloth (mesh opening 1.27 cm) approximately 10.5 cm in diameter was fitted inside the cap of the 3.8-liter jar. **Center:** Two 10-cm diameter ventilation holes were cut in opposing side walls of the 3.8-liter jar and the outside wall around each hole was scuffed with a wire brush to improve glue adhesion. Hot glue was then used to adhere a 12.5-cm diameter piece of fine polyester mesh (435 µm opening, product EX-40-200, NBC Meshtech Americas, Batavia IL, USA) over each hole. A 10.5-cm diameter hole was cut in the center of the base end (which was oriented upward) of the 3.8-liter jar and the outside wall around the hole was scuffed with a wire brush. **Top:** A clear, round plastic funnel (12 cm large end diameter, HNBun, UPC 696629698126, www.amazon.com) was adhered over the 10.5-cm hole of the 3.8-liter jar using hot glue. A 23-mm diameter hole was drilled through the center of the plastic (PP) cap of a 118-ml polystyrene wide-mouth jar (product S-9934, Uline, Pleasant Prairie, WI, USA). The neck of the funnel was pushed through the hole and secured in place with hot glue. All jars were screwed onto their respective caps. The cumulative per-unit cost of the nine components (tools and hot glue excluded) in June 2021 was $9.54 USD, most of which ($7.79 USD) was comprised by the 500-ml jar.

Evaluating the Container

Trial 1. The efficacy of the Lari-Leuco container for collecting *La. nigrinus* larvae and *Leucopis* spp. adults from hemlock foliage samples was evaluated in two trials. Trial 1 assessed a preliminary version of the container that lacked the inverted funnel leading to the top jar. In the preliminary design, a 48-mm hole was cut in the screw cap of the 118-ml polystyrene jar, and the cap and jar were imbedded directly into the upward-oriented base of the 3.8-liter jar and secured with hot glue (Fig. 1B). *Tsuga heterophylla* branches heavily infested with *A. tsugae* were clipped with a pole pruner in early February 2021 from three sites in the Puget Sound area of Washington, USA: Cavalero, Camano Island (CAV, 48.1691°N, −122.4813°W), Mabana Chapel, Camano Island (MAB, 48.0889°N, −122.4009°W), and Point Defiance Park, Tacoma (PDF, 47.3043°N, −122.5331°W). Branches were double-bagged and shipped overnight in tightly sealed boxes to the Sarkaria Arthropod Research

Laboratory (SARL) quarantine greenhouse, in Ithaca, NY, USA (USDA APHIS permit P526P-18-00945). The greenhouse had natural lighting and was maintained at 12.8–18.3°C.

In quarantine, bulk foliage was cut into 25-cm long branches and arranged into bouquets of six branches. Five bouquets from each of the three field sites (15 total) were made by inserting cut ends of the 25 cm branches into floral foam that had been pressed into a 60-ml polystyrene, open-topped, straight-sided cup (product S-12752, Uline, Pleasant Prairie, WI, USA), submerged in tap water overnight, and covered with laboratory film (Parafilm M, Bemis Manufacturing Company, Sheboygan Falls, WI, USA). The number of *A. tsugae* ovisacs per cm of shoot growth was counted on one 25-cm branch per bouquet. Each bouquet was placed on the steel hardware cloth platform within a Lari-Leuco container. Lari-Leuco containers with foliage were then placed within custom fabricated acrylic cages (Leigh-Dale Specialties, Syracuse, NY, USA) with 120-μ mesh (Component Supply Co., Sparta, TN) (Dietschler et al. 2021). Lari-Leuco containers were checked every 1–2 d and overhead grow lights mounted above the cages were turned on 10-20 min prior to each check to help aggregate adult insects toward the top of the containers. At each check, the number, life stage, and specific location of all *Laricobius* or *Leucopis* spp. that had dispersed from the foliage were recorded. Locations within the Lari-Leuco container were classified as either on target (i.e., within the top collection jar for *Leucopis* adults, or the bottom collection jar for *Laricobius* larvae) or off target (on the sides, ceiling or corners of the central jar, or any other off-target location). Regardless of location, all *Laricobius* or *Leucopis* spp. were removed from the container on each check and used for other laboratory research. Containers in Trial 1 were monitored from 9 February to 1 March 2021, when the branches began to show signs of desiccation due to inadequate saturation of the floral foam.

Trial 2. A second trial was conducted from 2 March to 29 March 2021 to correct for inadequate floral foam saturation, allow for more *Leucopis* adult emergence, and evaluate a structural modification to the preliminary container design. Methodology for Trial 2 was the same as in Trial 1 with the following exceptions. Fresh infested foliage was collected from only two sites (CAV and MAB) and this material was used to create eight bouquets from each site. When creating bouquets, floral foam blocks (5.5 × 4.5 × 4.0 cm) were submerged directly (without use of a cup) in tap water overnight and wrapped with laboratory film. This change was made because the cups used in Trial 1 tended to trap air and invert when submerged, resulting in incomplete saturation of the foam. For each site, four bouquets were randomly assigned to each of two Lari-Leuco container top designs: no funnel (the preliminary design used in Trial 1) and funnel (the addition of a funnel leading to the top collection jar, Fig. 1A).

Statistical Analyses

Each Lari-Leuco container was considered as an experimental unit. For each trial, the mean (SE) density of *A. tsugae* (ovisacs/cm) on the foliage was calculated, and the mean (SE) and total number of *Laricobius* larvae and *Leucopis* adults collected were calculated. In Trial 2, logistic regression was used to evaluate whether container top design (funnel vs. no funnel) affected the proportion of *Leucopis* adults recovered in the target collection jar. Each adult *Leucopis* observation was coded as a binary variable (on vs. off target) and this was modeled using a generalized linear mixed model with binomial distribution, with top design as a fixed effect and source field site of the foliage as a random effect. Analyses were conducted using JMP 14.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

Trial 1. In Trial 1, the mean (SE) density of *A. tsugae* on the source foliage was 1.8 (0.2) ovisacs/cm (Table 1). On average, more than 11 *Laricobius* larvae, but fewer than one *Leucopis* adult, were recovered per container (Table 1). Of the 174 *Laricobius* larvae that were recovered from all containers, 100% of these were located in the target (bottom) collection jar (Table 2). Larval drop per container over time in Trial 1 followed a relatively unimodal distribution, beginning on day nine of the experiment (17 Feb), peaking at 4.4 larvae/day on day 13 (21 Feb), and tapering to near zero by the end of the experiment on day 21 (1 Mar; Fig. 2). Only six *Leucopis* adults were recovered in Trial 1, 50% of which were collected from the target (top) jar (Table 2). This limited *Leucopis* adult emergence generally coincided with the onset of *Laricobius* larval drop but ceased after day 11 (19 Feb; Fig. 2).

Trial 2. In Trial 2, the mean density of *A. tsugae* on the hemlock foliage was similar to that observed in Trial 1 (Table 1). On average, about 21 *Laricobius* larvae and 8 *Leucopis* adults were recovered per container (Table 1). Of the 343 *Laricobius* larvae recovered from all containers in Trial 2, 100% of these were located in the target (bottom) collection jar (Table 2). *Laricobius* larval drop per container over time in Trial 2 was again relatively unimodal, beginning on day three of the experiment (4 Mar), peaking at 5.3 larvae/day on day 11 (12 Mar), and ending after day 18 (19 Mar) (Fig. 2). A total of 124 *Leucopis* adults were recovered in Trial 2 (Table 1). Container top design had a significant effect on the proportion of *Leucopis* adults recovered in the target (top) collection jar ($F = 14.5$, df = 1, 119, $P < 0.001$). Adding a funnel that led to the top collection jar significantly increased the proportion of *Leucopis* adults recovered in the target jar from 60 to 94% (Table 2). In Trial 2, limited *Leucopis* adult emergence coincided with the onset of *Laricobius* larval drop, but numerous *Leucopis* emerged after *Laricobius* larval drop in a pulse that began on day 16 (17 Mar), peaking at 1.7 adults/day on day 21 (22 Mar) tapering to near zero by the end of the experiment on day 28 (29 Mar; Fig. 2).

Discussion

The Lari-Leuco container with the improved top design was effective for collecting descending *Laricobius* larvae and ascending *Leucopis* adults dispersing from the same *T. heterophylla* foliage sample. All *Laricobius* larvae that dropped from the foliage after feeding on their *A. tsugae* prey were easily recovered from the target (bottom) collection jar. Although the preliminary container design (Fig. 1B)

Table 1. Mean (SE) number of hemlock woolly adelgid (HWA) ovisacs/cm, *Laricobius* spp. larvae recovered, and *Leucopis* spp. adults recovered from containers with *Tsuga heterophylla* branches during 9–28 Feb (Trial 1) and 2–29 Mar (Trial 2) 2021

Trial	No. of containers	HWA ovisacs/cm	*Laricobius* larvae/container	*Leucopis* adults/container
1	15	1.8 (0.2)	11.6 (2.3)	0.4 (0.1)
2	16	1.5 (0.1)	21.4 (3.5)	7.8 (1.1)

Table 2. Total number and percentage of *Laricobius* spp. larvae and *Leucopis* spp. adults recovered relative to the intended target location (on vs. off) in containers with *Tsuga heterophylla* branches during 9–28 Feb (Trial 1) and 2–29 Mar (Trial 2) 2021

			Laricobius larvae			*Leucopis* adults		
Trial	Top design	No. of containers	On target	Off target	% On target	On target	Off target	% On target
1	No funnel	15	174	0	100.0	3	3	50.0
2	No Funnel	8	144	0	100.0	33	22	60.0
	Funnel	8	199	0	100.0	65	4	94.2

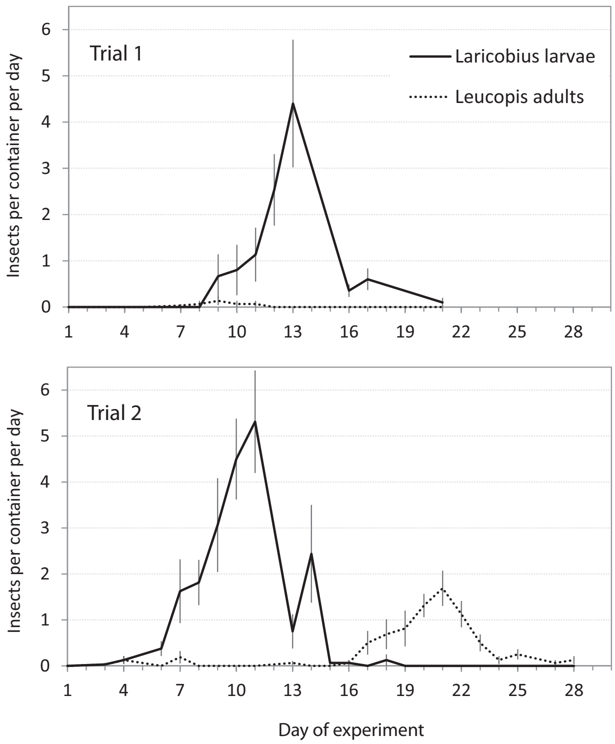

Fig. 2. Mean number of *Laricobius nigrinus* larvae and *Leucopis* spp. adults collected per container per day from *Tsuga heterophylla* foliage in Trial 1 (top) and Trial 2 (bottom), 9 Feb–29 Mar 2021.

successfully captured only 50% to 60% (Trials 1 and 2, respectively) of the *Leucopis* adults in the target collection jar, addition of an inverted funnel leading to the top jar (Fig. 1A-7) increased the target capture rate to 94%. These results demonstrate that the Lari-Leuco container efficiently separates these two groups of predatory insects from their prey's host foliage into removable jars where they are easily seen and obtained as live specimens.

The basal half of the Lari-Leuco container features a slightly tapered neck on the 3.8-liter central jar (Fig 1A-3) which functions as a simplified Berlese-style funnel (Schauff 2001) by channeling falling larvae through the hardware-cloth foliage platform (Fig. 1A-5) and into the bottom 500-ml jar (Fig. 1A-1). This design is similar to the collection funnel described by Salom et al. (2012) for mass-rearing *La. nigrinus*, which funnels larvae descending from a larger foliage chamber (approximately 35 liters) into a smaller (236 ml) removable jar. Although the design of Salom et al. (2012) is advantageous for mass rearing *Laricobius* from large volumes of hemlock foliage (Foley et al. 2021), the funnels must be suspended on custom-built racks to remove the bottom jars. In contrast, the bottom 500-ml jar on the Lari-Leuco container is wide enough for the entire apparatus to stand freely on a benchtop, and the remainder of the container also stands freely when the bottom jar is temporarily removed to access the insects. Thus, the Lari-Leuco containers require less space and material than the mass-rearing funnels, and are advantageous when monitoring numerous, smaller foliage samples that must be tracked independently, such as in replicated research designs or when monitoring multiple sites or trees for predator establishment.

The efficacy of the Lari-Leuco container for collecting *Leucopis* spp. silver flies was significantly improved by replacing the ceiling with an inverted plastic funnel. In the preliminary (no funnel) design, the interior ceiling of the 3.8-liter jar was slightly convex, creating a rounded 'corner' that was a few millimeters higher than the entrance to the top collection jar (Fig 1B). Of the 25 silver flies (in both trials combined) that did not reach the top collection jar of the preliminary 'no funnel' design, most of these (56%) were found in the ceiling corner of the central jar. This suggests that *Leucopis* adults are not only positively phototactic (moving toward the lights mounted above the containers) but also somewhat negatively gravitactic (less likely to descend while walking at a localized maximum elevation). Live flies were also recovered occasionally on the central jar side walls and in the bottom (non-target) collection jar, indicating that *Leucopis* adult exploration of the container was not strictly in directional response to light and gravity. Nonetheless, the inverted funnel in the final design (Fig. 1A) largely eliminated this ceiling corner and created a more continuous upward slope from the foliage chamber to the target jar, increasing the target collection rate to 94%. The extension of the narrow end of the funnel into the top collection jar also likely helped to keep flies from escaping the collection jar once inside it.

Laboratory collection of adult *Leucopis* predators from HWA-infested foliage is commonly performed within single-chambered, tent-style cages covered with fine mesh or other similar, custom-built structures (Motley et al. 2017, Neidermeier et al. 2020). These types of cages have also been used to collect *Leucopis* adults and *Laricobius* larvae simultaneously (Dietschler et al. 2021). When these cages are used to collect *Leucopis*, the adults must be aspirated from different locations on the ceiling or walls of the foliage chamber, typically by inserting one's hand and the aspirator tube through a mesh sleeve opening. Similarly, *Laricobius* larvae collected from such cages must be obtained individually from the floor of the cage, possibly requiring movement of the foliage bouquet to see them. The Lari-Leuco container design overcomes these practical limitations by isolating the insects into jars that can be removed and sealed without having to access the foliage chamber nor aspirate/collect insects individually.

The temporal patterns of *Laricobius* larval drop and *Leucopis* adult emergence observed in this evaluation (Fig. 2) were consistent with those described by Dietschler et al. (2021) using *T. heterophylla* foliage obtained from the same geographic region. As HWA sistens adults matured and began to oviposit in early spring, Dietschler et al. (2021) observed a consistent pattern in which adult *Le. argenticollis* emerged first, followed by *La. nigrinus* larval drop, followed by adult *Le. piniperda* emergence, and lastly by a second emergence of *Le. argenticollis*. Thus, although *Leucopis* adults were not identified to species in this evaluation, the few *Leucopis* that emerged near the onset of *La. nigrinus* larval drop in Trials 1 and 2 likely represented the end of the first *Le. argenticollis* emergence, whereas the strong *Leucopis* emergence peak that followed *La. nigrinus* drop in Trial 2 (Fig. 2) was likely *Le. piniperda*. These results indicate that the container is effective for collecting adults of both species of *Leucopis*. It is presumed that Trial 1 concluded before the onset of *Le. piniperda* emergence, and Trial 2 concluded before a second *Le. argenticollis* emergence began.

Finally, although the distinct peak in *Laricobius* larval drop was observed in both trials during different calendar dates, this was not due to two natural peaks in the *La. nigrinus* larval population; rather, it was due to bringing foliage from cool field conditions of the Puget Sound area into the warmer temperature conditions of the laboratory (which stimulated larval drop) at two different times. *Laricobius* larval drop in the laboratory began about 6 d earlier in Trial 2 compared to Trial 1; this was due to the fact that the Trial 2 foliage was collected about 3 wk later than in Trial 1, during which additional field development of larvae occurred. The warmer conditions of the laboratory (range 12.8 to 18.3°C) likely accelerated the onset of larval drop relative to what could be expected in the field (range: –4.4 to 12.2°C through February 2021). In the laboratory, *Laricobius* larval drop lasted 13–16 d, and the second *Leucopis* adult emergence (likely *Le. piniperda*) lasted approximately 12 d.

In conclusion, the Lari-Leuco container introduced and evaluated here is a potential research and monitoring tool to benefit the integrated pest management program for HWA on hemlock in eastern North America. The containers can be used to help monitor for establishment of both *Laricobius* and *Leucopis* predators from the same hemlock foliage samples clipped in the early spring when they are infested with HWA sistens adults. The container's compact size and low cost of construction (<$10 USD in component materials) also make it useful for conducting research on both predator genera when sample replication is a priority. Although the container was designed and tested specifically for use in HWA biological control, it could also prove useful in the study of other insect-plant or predator-prey systems in which simultaneous separation of ascending and descending insect life stages is desired.

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