GUIDELINES FOR REARING *LARICOBIUS NIGRINUS* FENDER

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ABSTRACT

Laricobius nigrinus (Coleoptera: Derodontidae) is attracting attention for its potential usefulness as a predator of the hemlock woolly adelgid (HWA), *Adelges tsugae Annand*. *Laricobius nigrinus* is a univoltine species with an unusual lifecycle, where adults spend the summer in diapause but are active for the rest of the year. Field and laboratory studies indicate *L. nigrinus* possesses many characteristics deemed favorable for biological control agents. To expedite its establishment and be a viable control option for HWA, *L. nigrinus* must be reared in large numbers for field releases. Procedures that have shown some success in laboratory rearing of *L. nigrinus* at Virginia Tech are described.

KEYWORDS

Hemlock woolly adelgid, biological control, colony rearing, predators.

INTRODUCTION

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae) is an exotic insect that attacks and kills eastern hemlock, *Tsuga canadensis* (L.) and Carolina hemlock, *T. caroliniana* Engelmann trees in the eastern United States (Ward et al. 2004). HWA is an innocuous inhabitant of *Tsuga* spp in Asia, where it is believed to originate (Montgomery et al. 2000). Populations of this insect may be regulated by host resistance and natural enemies (McClure et al. 2000). In eastern North America, HWA populations rapidly reach lethal levels and with few chemical control options, natural enemies may be the most promising method for controlling HWA in the eastern states (Wallace and Hain 2000).

Laricobius nigrinus Fender (Coleoptera: Derodontidae) is a predator found in association with HWA in western North America (Zilahi-Balogh et al. 2003a), where hemlock is not typically injured by HWA (Furniss and Carolin 1977). *L. nigrinus* is being evaluated for its potential as a biological control agent for HWA. Field and laboratory studies have revealed that *L. nigrinus* can play an important role as part of a complex of biological control agents aimed at regulating HWA abundance.

This predator has several attributes that are considered necessary for a successful biological control agent including: synchrony and adaptability with its environment and host, host-searching capacity, ability to increase in numbers; and general mobility (Huffaker and

Kennett 1969). Zilahi-Balogh (2001) concluded that *L. nigrinus* adults and larvae feed selectively on HWA and in larval development tests, *L. nigrinus* completed development only on HWA, indicating that it is unable to survive on other prey species (Zilahi-Balogh et al. 2002). Field studies in British Columbia, Canada have shown that its lifecycle is highly synchronous with HWA, and field studies in Virginia show similar host synchrony in the eastern U.S. (Lamb et al. 2005). In addition, two years after a small field release of *L. nigrinus* in Virginia, F_2 adults were recovered, indicating their potential ability to establish in the eastern U.S. (Lamb et al. unpublished data).

To be a viable biological control candidate, *L. nigrinus* must be successfully mass reared for field releases. Our mass rearing efforts have focused on laboratory propagation and the use of field insectaries. Successful procedures for laboratory rearing *L. nigrinus* have been developed after four years of research. Although there is room for improvement, the following methods have yielded several generations of the beetle. This paper describes the life cycle of *L. nigrinus* and current procedures used for rearing each life stage in the laboratory at Virginia Tech.

LIFE CYCLE OF *LARICOBIUS NIGRINUS* AND *ADELGES TSUGAE*

Field studies in British Columbia (Zilahi-Balogh et al. 2003a) and rearing *L. nigrinus* in the laboratory have revealed a univoltine life cycle that is synchronous with the life cycle of HWA (Zilahi-Balogh et al. 2003b). HWA has a complex polymorphic life cycle that involves two asexual generations annually (McClure 1991). The overwintering generation (sistens) feeds and develops throughout the fall and winter months and deposits eggs within woolly ovisacs in early spring. The second generation (progrediens) lays eggs within woolly ovisacs in late spring, which hatch into sistens crawlers that settle at the base of young needles, feed for a short time, and then enter aestivation (summer diapause) (McClure 1989; Gray and Salom 1996; Salom et al. 2002). Aestivation lasts for several months, and sistens nymphs resume feeding and development in October (McClure 1987; Gray and Salom 1996).

L. nigrinus adults overwinter on the hemlock branches, feeding on HWA sistens. In late January or early February, adult *L. nigrinus* begin ovipositing single eggs directly in sistens ovisacs. Oviposition by *L. nigrinus* is synchronous with the oviposition period of HWA sistens, beginning in January or February and ending in May or June. Eggs hatch and eclosed larvae feed on HWA eggs. Larvae develop through four instars and the mature larvae drop to the ground and migrate to a pupation site within the soil. Development from egg to prepupae takes five to six weeks using the procedures outlined below. At 15°C, *L. nigrinus* remain as prepupae for about 10 days prior to pupation (Zilahi-Balogh et al. 2003b). Pupal development occurs in cells in the soil, and after about two weeks, pupae develop into adults. The adults remain in the soil and enter aestival diapause in early summer. In October, adult *L. nigrinus* emerge from the soil and migrate back to hemlock trees in search of HWA. This is approximately the same time that the HWA sistens are resuming development. Thus, both species enter and complete aestival diapause at approximately the same time.

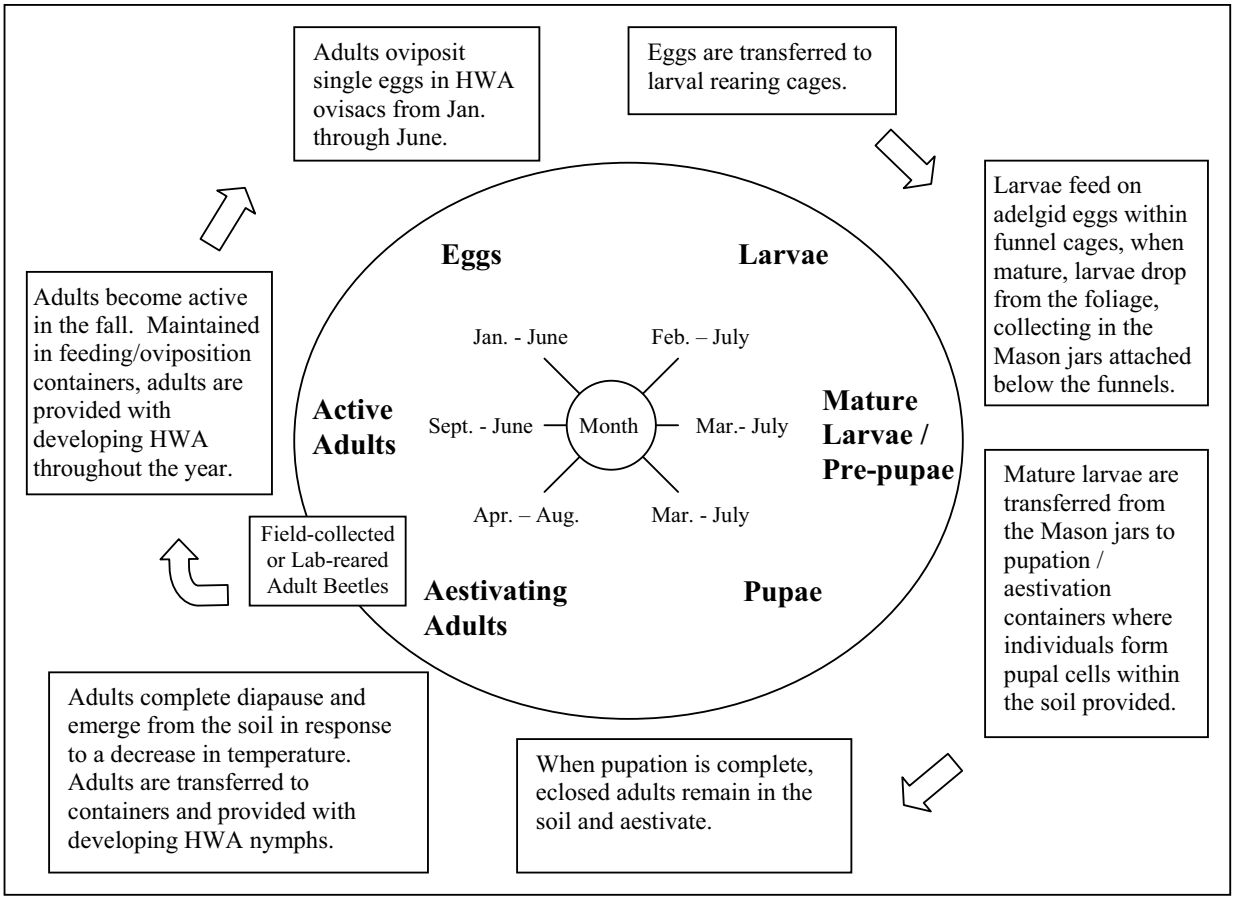

Figure 1. A representation of the univoltine life cycle of *Laricobius nigrinus* and the primary procedures occurring during the rearing of each life stage.

Table 1. Holding temperatures and day-length for *L. nigrinus* adults at different times of their active period.

Time of Year	Day:Night Temp. (°C)[1]	Daylength (h)[2]	Adults per Container	Frequency of Feeding	Destination of Old Hemlock Bouquets
Pre-oviposition (Oct.-Jan.)	4:2	12 to 10	50	Every 2 weeks	Large plexiglass box to recover lost adults
Oviposition (Feb.-Mar.)	6:4	10 to 12	25	Once a week	Inserted in floral foam and placed in funnel cages
Oviposition (Apr.-Jun.)	10:8	12 to 16	25	Twice a week	Inserted in floral foam and placed in funnel cages

[1] Increase in temperature should coincide with peak L. *nigrinus* oviposition and when HWA ovisacs are 50-75% full.
[2] Daylength is gradually increased or decreased over each period.

Figure 1 represents the life cycle of *L. nigrinus* and the general rearing procedures coinciding with each life stage. References made to specific months indicate the average time of year HWA is available in particular stages in Blacksburg, Virginia. The exact time of year *L. nigrinus* begins and ends oviposition is influenced by the stage of the HWA provided to them.

FEEDING *L. NIGRINUS* ADULTS (EARLY FALL THROUGH LATE SPRING)

Laricobius nigrinus adults become active in the early months of fall and require host material upon emergence. They begin feeding on HWA nymphs attached to hemlock branches and feed all winter. In late January, they begin to lay eggs, singly in the HWA wool sacs (ovisacs) and continue until about June (peaks in late March–early April).

The general procedures for storing and maintaining adults are similar throughout the year, however there are minor changes in conditions between the pre-oviposition (Oct.-Jan.) and oviposition (Feb-June) periods (Table 1).

Storage (Adult Feeding/Oviposition Containers)

- Gallon-sized plastic containers (screened holes on the side and the lid) with moistened filter paper cut to fit the bottom.
- Each container holds one hemlock "bouquet" (a floral foam-filled film canister holding 10-15 heavily HWA-infested hemlock branches (approximately 20 cm long).
- Adults are stored in environmental chambers programmed to create conditions shown in Table 1.

Maintenance

- At the end of each feeding period, every adult is recovered from its current container and transferred to a new container with a fresh hemlock bouquet (spray with distilled water).
- Containers are kept at 4°C immediately before adults are fed to reduce flight.
- Adults transfers are carried out under a nylon screen to a table-top cage to prevent flying adults from escaping.

(a) Pre-ovipositing Adults

– If all adults are recovered from old containers, discard the old hemlock bouquet.

– If some individuals are not accounted for, old hemlock branches are placed in a large plexiglass box at room temperature and observed daily for missing adults.

(b) Ovipositing Adults

– It is critical to provide heavily-infested foliage with ovipositing HWA to adults to ensure that early instars can access adelgid eggs. Adult *L. nigrinus* should be provided with enough adelgids (preferably excess amounts, ~100 ovisacs per adult beetle) for themselves and the early instars of the progeny they produce.

– To ensure predator eggs are deposited in optimal sites (singly, in full adelgid ovisacs) adults are fed twice a week and the number of adults per container is reduced (Table 1). These modifications also decrease larval competition during their development and increase synchrony of larval maturation.

– The hemlock branches from which adults are removed from at each feeding contain the *L. nigrinus* eggs laid since the time of the last feeding. These branches are transferred to parafilm-wrapped floral foam blocks and are inserted alternately with fresh hemlock branches infested with oviposting HWA. The additional HWA fresh foliage provides host material for the more mobile 3rd and 4th *L. nigrinus* instars. These blocks are placed into funnel cages (Lamb et al. 2002).

DEVELOPING *L. NIGRINUS* LARVAE (DEVELOPING FROM FEBRUARY TO JUNE)

After a few weeks, the *L. nigrinus* eggs hatch within the woolly ovisac and early instars feed on the nearby adelgid eggs. Third and fourth instars are more mobile and feed on adelgid eggs and developing nymphs. The duration for *L. nigrinus* development from egg to mature fourth instar larvae (pre-pupae) varies depending on temperature and food quality. The larvae develop at temperatures ranging between 9° and 21° C; however, pupal development does not occur at the higher temperatures of this range. The optimal temperature for successful development of larvae is likely between 12° and 18°C (Zilahi-Balogh et al. 2003b). After consuming a sufficient amount of prey (200+ eggs) to complete larval development, the prepupal stage is reached. This stage is a non-feeding fourth instar that drops from the hemlock branches and seeks a pupation site in the soil. Larvae are reared in funnel cages, designed to take advantage of this "dropping/migrating" stage so mature larvae (prepupae) essentially collect themselves.

Storage (Larval Funnel Cages)

- Hemlock branches with HWA and *L. nigrinus* eggs are inserted into parafilm-wrapped floral foam blocks, intermixed with fresh HWA-infested branches, and placed into funnel cages. A cylindrical top, made of .7 mm acetate, is placed over the 25 cm funnel base and a black, pint-size Mason jar is attached underneath the funnel. Each Mason jar contains about 2 teaspoons of moistened pupating medium.[1]
- The funnel cages are set up on racks in cold rooms that are maintained at about 13°C and daylength increased at a rate similar to natural conditions. At this temperature and with adequate prey, mature larvae begin collecting in the jars below the funnels after 3-4 weeks. If the temperature increases above 18°C, development rate of larvae increases dramatically, causing funnels to yield both mature and immature larvae. It is possible that the higher temperatures cause the larvae to be more active, resulting in immature

[1] Pupating Medium:
Southland peat moss (sifted through mesh screen with 0.5 cm openings), Mosser Lee long fiber sphagnum moss (ground finely), and Play sand.
Mix materials (2:2:1 peat:sphagnum:sand) and add distilled water until mixture reaches ~50% saturation. Mixture is then sterilized using two treatments of heat and pressure for several hours or steam sterilization for 12+ hours separated by 24+ hours at room temperature.

larvae collecting in the jars below the funnels. This situation can also be observed when larvae are not provided with an adequate amount of HWA eggs within the funnel. Differences in appearance and behavior can separate mature and immature larvae.[2]

Maintenance

- Funnels are set up immediately after adults are fed.
- The foliage in each funnel is sprayed with distilled water twice a month and soil in collecting jars below are moistened every couple of days (in anticipation for maturing larvae).
- Several days before anticipated larval drop, the collecting jars below the funnels are checked by emptying the contents into a Petri dish, and a paintbrush is used to sift through the materials to recover any larvae.
- Collecting jars are checked daily to separate mature from immature larvae; mature larvae become settled for pupation if given enough time (~36 hours), but mortality of immature larvae in the collecting jars is high unless immature larvae are returned to branches with prey promptly.
- Mature larvae (prepupae) are transferred, using a moistened paintbrush, to pupation/aestivation containers for the summer.
- Immature larvae are transferred to HWA-infested hemlock and placed back in a funnel cage.

MATURE LARVAE/PREPUPAE (MARCH – JULY)

Upon being transferred to pupation/aestivation containers, mature larvae burrow into the soil, form pupal cells, and remain in a c-shape within their pupal cells for 10-14 days before development into pupae. Within 36 hours of dropping from the foliage, prepupae will settle into cells within the soil to pupate. It is important that individuals are transferred to a pupation/aestivation container before a pupal cell is formed as they have difficulty moving to a new site to pupate after they have settled.

Storage (Pupation/Aestivation Containers)

- Quart-sized and half gallon-sized, clear and opaque, plastic containers with several screened holes for air movement are used as pupation/aestivation containers. Each container contains at least 5 cm of pupating medium; the bottom layer is packed tightly and the upper layer loosely.
- Depending on the size of the container, between 50 and 200 larvae are placed in each container.

[2] Mature larvae have a yellow ventral side and sclerotized dorsal side, typically are moving and are negatively phototactic and will drop off the branch if placed back on an HWA-infested hemlock branch.
Immature larvae are smaller in size, dark in color, and often have white wool attached to their dorsal side; attach themselves to objects and do not move; and will not drop off branch if placed back onto HWA-infested hemlock branch.

- Mold within the pupation/aestivation containers has been a recurring problem. Sources of fungal spores are minimized, all containers and the pupation medium are sterilized, and an anti-fungal agent (methyl paraben) is applied to the soil surface weekly.
- Pupation/aestivation containers are maintained at 15°C and long days (16 hours).

Maintainence

- Moisture level of pupation medium should be maintained at about 30-40% saturation by spraying distilled water into containers once a week.

PUPAE/AESTIVATING ADULTS (LATE APRIL THROUGH JULY)

The delicate pupae develop two weeks after burrowing in the soil. *L. nigrinus* pupae are bright yellow and require a narrow temperature regime during this time (Zilahi-Balogh et al. 2003c). At 15°C, pupation is complete in approximately two weeks and the newly eclosed adults remain under the soil surface, and enter summer diapause (aestivation). Adults remain in the soil until they are cued to emerge by a decrease in temperature (Lamb, unpublished data). To prevent premature emergence, aestivating adults are stored at high temperatures until mid-September, at which time the temperature is decreased, and adults emerge several weeks later.

Storage (Pupation/Aestivation Containers)

- Pupation/aestivation containers are kept in the large cold room at 15°C until pupation is complete. Once adults have eclosed, containers are moved to 19°C and long daylength (16 hours) until mid-September, when the containers are moved to 13°C and decreasing daylength at a rate similar to natural conditions.

Maintenance

- Pupation/aestivation containers are watered weekly to maintain about 30% soil saturation.
- Containers are checked every other day beginning in late August to recover adults emerging prematurely
- In September, after the temperature is decreased, containers are checked daily for emerging adults.

EMERGING ADULTS (SEPTEMBER – DECEMBER)

Adults complete aestivation and emerge from the soil in the fall, approximately three weeks after the temperature is decreased. Adults become active and climb up the sides and lids of the pupation/aestivation containers. Premature adult emergence is undesired because emerging adults require developing HWA nymphs to feed on, which do not emerge from diapause until October.

Storage (Feeding/Oviposition Containers)

- Emerging adults are transferred from the lids and sides of the pupation/aestivation containers to adult feeding/oviposition containers with developing HWA nymphs and distilled water.
- Recently emergent adults are kept at 6°C and 12 hours daylength. Temperature is lowered if prey is limited.

Maintenance

- Pupation/aestivation containers are checked for emerging adults several times per day, particularly in the late afternoon and evening.
- Distilled water is sprayed through the screen lids of the feeding/oviposition containers several times per week to ensure adults have adequate water. When temperature is decreased (about November), the frequency of watering decreases.
- Active adults are either released in the field or maintained in feeding/oviposition containers according to the procedures outlined in Table 1.

CONCLUSION

These rearing procedures have produced approximately 44,000 F_1 adults during the past 4 years. Survival through pupation and aestivation has a greater influence on the number of F_1 adults produced than size of the initial colony. In addition, field collected adults seem to be more fecund than lab-reared adults; therefore, starter colonies should be larger ($\geq$2000) if adults are lab-reared and smaller (500-1000) if adults are field-collected.

Several parts of the current rearing procedures need further improvement. Most importantly, the preparation and maintenance of the pupation medium needs to be modified to reduce the high level of pre-pupal and pupal mortality that occurs. In addition, pupation/aestivation containers should be modified to increase the ease and efficiency of adult recovery while they are emerging from the soil. Development of rearing containers that take advantage of the "migratory stage" of mature larvae immediately after dropping from the foliage would reduce the time spent checking funnel jars. The cage should allow larvae, needles, and debris to fall into one level and from there, enable the larvae to move themselves to a pupation/aestivation container, thereby preventing needles and debris from falling into the sterile pupating medium.

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