Field Evaluation and Improvement of Rearing Procedures for *Laricobius nigrinus* (Fender) (Coleoptera: Derodontidae), a Predator of Hemlock Woolly Adelgid

Ashley B. Lamb, Scott M. Salom, and Loke T. Kok

Virginia Polytechnic Institute and State University
216 Price Hall, Virginia Tech, Blacksburg, VA, 24061-0319

Abstract

Laricobius nigrinus (Coleoptera: Derodontidae) shows promise as a useful biological control agent for hemlock woolly adelgid, *Adelges tsugae* (Homoptera: Adelgidae). A field evaluation was conducted to assess the survival and reproduction of *L. nigrinus* under natural conditions and to quantify their impact on HWA populations. A predator exclusion study was conducted using 162 branches, each receiving one of three treatments: caged with predators, caged without predators, and uncaged branches. *L. nigrinus* adults survived from February to April, producing up to 41 progeny per female. Adelgid densities on branches exposed to *L. nigrinus* suffered a significantly higher rate of decrease than branches not exposed to predators.

Current rearing of *L. nigrinus* is constrained by high mortality and a lack of knowledge as to which life stages incur significant mortality. A new rearing cage has been devised that enables the quantification of larvae reaching maturity, of pupal survival, and survival through aestivation. Our objective was to streamline rearing procedures by identifying life stages with high developmental mortality and to determine factors critical to the success of each life stage. Factors investigated were optimal larval density per cage and moisture level of soil in which adults aestivate. Density of individuals per cage did not significantly affect larval survival between 10 to 50 eggs per cage. Egg and larval stages suffered an average of 28% mortality at the egg densities tested. Survival through aestivation was significantly higher in the high and medium soil moisture levels than at the low moisture level. The *L. nigrinus* colony suffered approximately 30% mortality during pupation and approximately 60% during aestivation. Future research will focus on identifying other factors affecting *L. nigrinus* survival through aestivation.

Keywords:

Laricobius nigrinus, Derodontidae, Biological Control, *Adelges tsugae*, Field Evaluation, Rearing Procedures.

Introduction

Hemlock woolly adelgid (HWA), *Adelges tsugae* Annand (Homoptera: Adelgidae) is an exotic pest that attacks and kills hemlock trees in the eastern United States. It currently infests hemlock in more than 30% of its geographic range and continues to grow with potential to spread throughout its entire native range (McClure 1996). Preservation of hemlock forests is important ecologically as they provide critical and unique habitat for many bird, mammal, amphibian, and fish species (Evans et al. 1996).

Due to the apparent lack of natural enemies of HWA in the eastern United States, classical biological control has become the most promising control option for this pest (Wallace and Hain 2000). It will likely require a complex of predators to effectively reduce adelgid populations below injurious levels. One predator that is currently being evaluated as a biological control agent is *Laricobius nigrinus* (Fender) (Coleoptera: Derodontidae). This predator can be found feeding on HWA in Washington, Oregon, Idaho, and British Columbia.

Field studies in British Columbia and investigations in the laboratory have revealed that *L. nigrinus* possesses many important qualities of a successful biological control agent. *Laricobius nigrinus* feeds selectively on HWA and is unable to complete development on any other insect (Zilahi-Balogh 2001). In addition, the life cycle of *L. nigrinus* is phenologically synchronous with the life cycle of HWA. Furthermore, *L. nigrinus* will likely be a complementary component of a predator complex because, being a winter-active predator, it is unlikely to compete with other predators that become active later in the spring. For this predator to be a viable biological control candidate, it must survive under natural field conditions and reduce the density of HWA within its feeding area.

Measuring the efficacy of a biological control candidate is an essential phase in a biological control program. The most convincing means of documenting the effect of a predator is by using check methods, where the predator is excluded from prey on certain branches of the host plant, while including them on other branches (Huffaker and Kennett 1969). A caged field experiment was conducted to determine the ability of *L. nigrinus* to survive in Virginia's climate and evaluate its impact on HWA populations.

In order to be a viable biological control agent, *L. nigrinus* must be reared in large numbers on a continuous basis for field evaluations and mass releases. A *L. nigrinus* colony has been maintained at Virginia Tech for four years, however, it has been constrained by high mortality rates. The development of more efficient rearing techniques is necessary for the mass production of this insect. *Laricobius nigrinus* has a complicated lifecycle, in which it remains on hemlock branches from the fall through the spring and aestivates in the soil throughout the summer. A better understanding of the physiological requirements necessary for its development will enhance the rearing capacity of this predator.

Our objective is to streamline rearing procedures for *L. nigrinus* by identifying life stages with high developmental mortality and determining factors critical to the survival of each life stage. A new rearing cage has been devised that enables the count of larvae reaching maturity and an estimate of pupal and aestivational success. Two experiments were conducted in the laboratory to determine the optimal larval density per cage and soil moisture level during aestivation.

Methods and Materials

Field Evaluations. A caged field study was conducted at three sites from February through June, 2001. The sites were located in southwest Virginia at Poverty Creek (Universal Trans-Mercator (UTM): 542429 N, 4123129 E), Big Stoney Creek (UTM: 533985 N, 4140859 E), and Mt. Lake Nature Conservancy (UTM: 541017 N, 4134851 E). Two trees were chosen at each site. Twenty-seven branches, heavily infested with HWA, were selected from each tree. The total numbers of adelgids were counted on the terminal 45 cm of each branch. Each branch was then randomly assigned one of three treatments: caged branches with two female adult *L. nigrinus*, caged branches without predators, and uncaged branches without predators. The latter two treatments served as controls. The cages consisted of sewn, fine-mesh sleeves (open at one end) that were attached to the terminal end of each branch with wire.

One replicate of each treatment was removed from each tree every two weeks and the total number of surviving predators, both adults and progeny, were tallied. In addition, the total number of surviving adelgids on each branch was determined. A two-factor analysis of variance was used to determine if *L. nigrinus* had a significant impact on the rate that adelgid densities decreased during each sample period.

Improving Rearing Procedures. Two experiments were conducted to determine the optimal larval density per rearing cage and the optimal soil moisture level for survival through aestivation. In addition, a new larval rearing cage was devised that enables us to count the number of larvae as they mature (Figure 1). The top section of the cage is an acetate cylinder with fine mesh screening on top. There is a coarse metal screen placed horizontally near the bottom of the cage, where floral foam with HWA-infested hemlock twigs and *L. nigrinus* eggs are placed. The bottom of the cage is a funnel screwed to collecting jars. Infested hemlock branches are added weekly to allow larvae to feed and develop until they are ready to drop to the soil. Mature larvae are collected and counted as they migrate down to the soil in search of a pupation site. Collection of *L. nigrinus* at this stage enables us to estimate egg and larval survival. In addition, we are able to calculate their survival through pupation and aestivation.

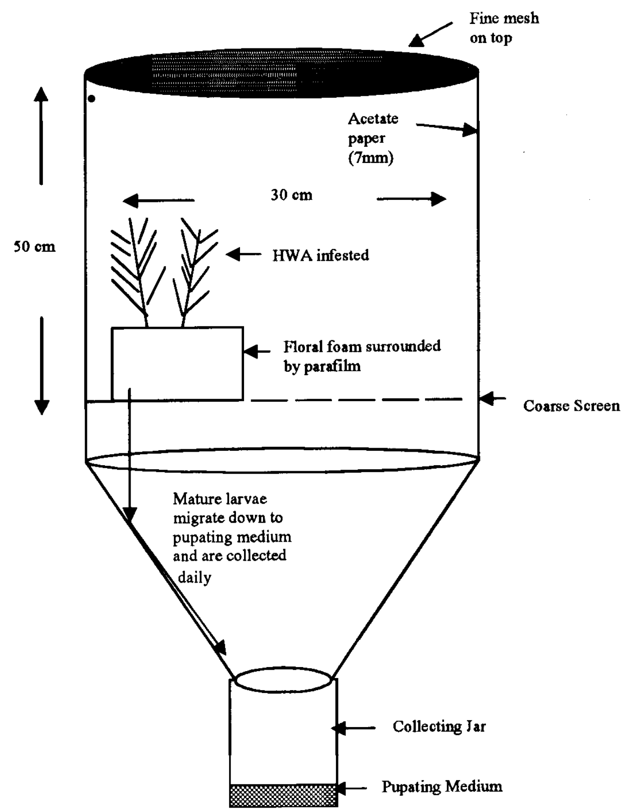

Figure 1. Illustration of the new rearing cage designed for rearing *L. nigrinus* eggs and larvae.

To determine the optimal larval density, 10, 20, 30, 40, and 50 eggs were placed in larval rearing cages with an adequate amount of prey. The numbers of larvae reaching maturation at each density were quantified. A one-way analysis of variance was used to determine whether the egg densities tested influenced larval survival.

The effect of soil moisture on the survival of aestivating adults was investigated by placing 10 mature larvae in each of 48 pint-sized plastic containers. Each of the containers had 2 cm of soil (3:2 mixture of potting soil:peat) and were maintained at one of three moisture levels (% saturation): high (35 to 45%), medium (20 to 25%), or low (5 to 10%). The number of adults emerging from aestivation at each soil moisture level were compared using a one-way analysis of variance.

Results and Discussion

Field Evaluation. *Laricobius nigrinus* adults survived and were reproductive from February through March of the study period (Figure 2). Adult survival and oviposition rate declined over time, likely due to a shortage of prey inside the cages. Adult *L. nigrinus* oviposited throughout March and April. Figure 1 indicates the average number of progeny per beetle at each sample period.

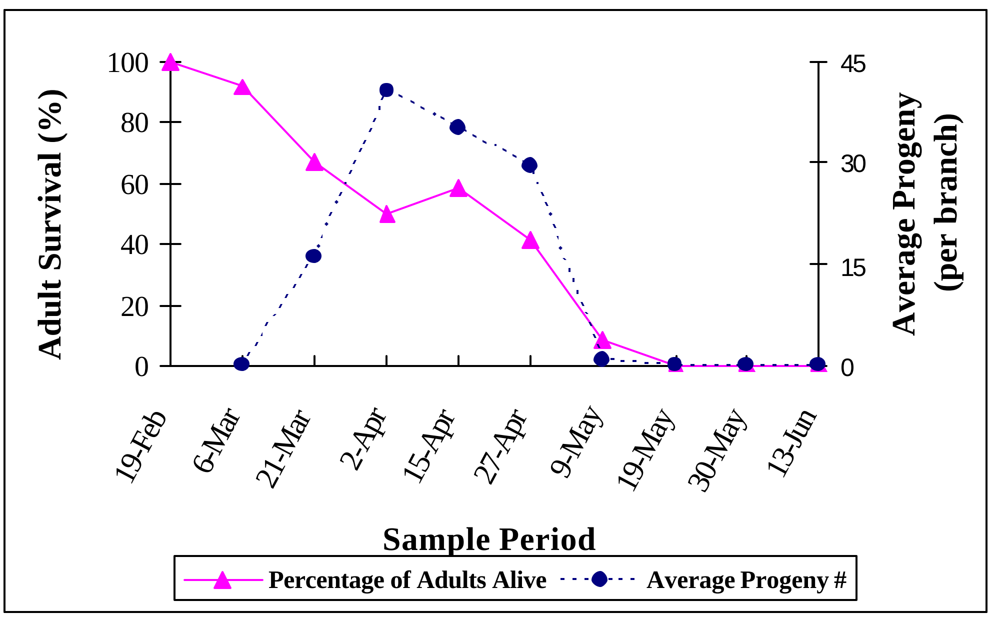

Figure 2. Percentage of *L. nigrinus* adults (maximum per sample period is 12) and the average number of progeny found alive at each sample period (N = 54). Adults were placed in sleeve cages on February 20, and sampled for the first time on March 7.

The branches exposed to *L. nigrinus* suffered a significantly higher rate of adelgid density decrease than branches not exposed to predators ($F_{(2,80)} = 59.14$, $p < 0.0001$). In six of the first seven sample periods, caged branches with *L. nigrinus* had a significantly higher rate of adelgid density decrease than caged branches without predators (Figure 3). In sample periods eight and nine, there was no difference in the rate of adelgid density decrease between the treatments. Throughout most of the study, there were few differences in the rate of adelgid density decrease between the two control treatments: caged and uncaged branches without predators. The decrease in adelgid densities per branch indicates that one female *L. nigrinus* consumed an average of nine adult adelgids per day from February through April.

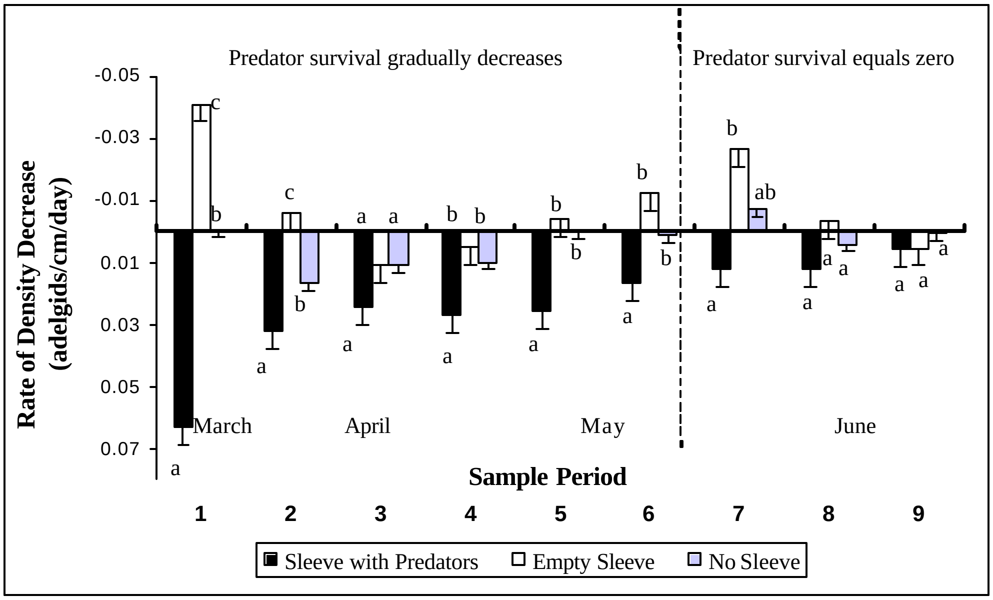

Figure 3. Mean mortality of adelgids in each treatment averaged across all sample periods in the field evaluation. Error bars indicate the standard deviation and different letters indicate a statistically significant difference between treatments at each sample period (N = 162).

The steady decline in the rate of adelgid density decrease can be attributed to two factors. First, the rate of adelgid density decrease is calculated by dividing the adelgid density by the number of days the branches are exposed to predators. Therefore, the adelgid densities in sample periods eight and nine were divided by 100 and 114 days respectively, as compared to 14 days in the first sample period. As a result, the rate of density decrease in sample periods eight and nine tend to be smaller. Also, there were no surviving *L. nigrinus* in the cages at this time and therefore there was no further decrease in adelgid density. Secondly, during the sample periods 8 and 9, the adelgid progediens generation had hatched, thereby increasing the adelgid density on branches.

With adequate prey, it is likely that *L. nigrinus* will survive longer, produce a greater number of progeny, and consume more adelgids. This will be investigated further during the second year of the field evaluation (fall/winter 2001-2002).

Improving Rearing Techniques. Egg and larval stages suffered approximately 30% mortality when given a sufficient amount of quality prey (800 linear cm of infested hemlock branches at a density of 2 adelgid ovisacs/cm) (Figure 4). The five egg densities tested did not significantly affect

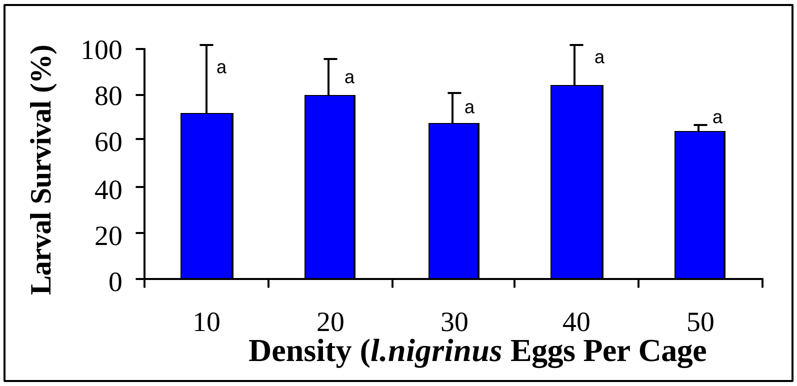

Figure 4. Mean survival of *L. nigrinus* larvae starting at different egg densities within a cage. Error bars represent the standard deviation. There is no significant difference in larval survival at the egg densities (mean = 72%) ($F_{(4, 15)} = 0.9$, $p = 0.49$).

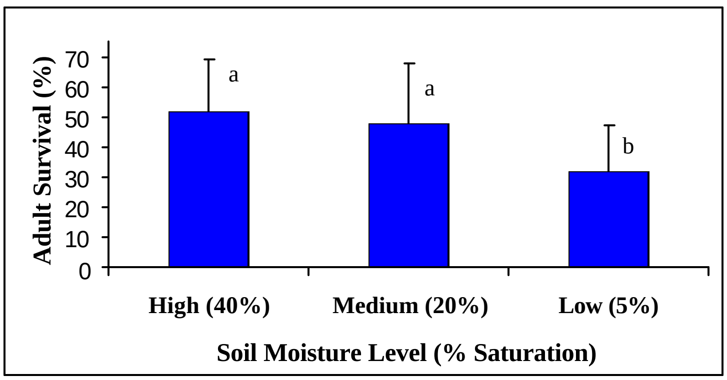

Figure 5. Mean survival of *L. nigrinus* adults through aestivation at high, medium, and low moisture levels. The error bars represent the standard deviation. There was significantly higher survival through aestivation at the high and medium moisture levels (52% and 48%) than at the low soil moisture level (32%) ($F_{(2, 45)} = 6.02$, $p < 0.005$).

larval survival. Adult survival through aestivation was significantly higher in the soils maintained at high and medium moisture levels (40% and 20% saturation) than in soil maintained at the low soil moisture level (5% saturation) (Figure 5).

In addition to testing larval density and soil moisture level, the entire *L. nigrinus* colony was monitored carefully throughout the year. The new rearing cages enable the number of larvae reaching maturity to be counted and an estimate of survival through pupation and aestivation can be made. By monitoring each life stage, we were able to identify the mortality rates at each stage. Success in rearing *L. nigrinus* from October 2000 to September 2001 is summarized in Table 1.

Table 1. Summary of the Number of Individuals at Each Life Stage of the *Laricobius nigrinus* Colony Between January 2001 and November 2001.[1]

L. nigrinus Life Stage	Estimated Mortality	Estimated Total
F1 Adults	(collected in Jan 2001 from British Columbia)	350 reproductive adults
Larvae	28%	7000
Pupae	31%	5000
F2 Adults (post-aestivation)	60%	1867

[1] An estimate of the total individuals and the overall mortality rate at each life stage is shown.

Between February and June 2001, a total of 7,000 larvae (28% mortality) were successfully reared to maturity from an initial 350 adults. Approximately 31% of these individuals did not complete pupation. A total of 1,867 adults emerged from aestivation, indicating the overall mortality during aestivation was approximately 60% Although this year has been more successful in rearing *L. nigrinus*, the colony continues to suffer high mortality rates. Current production of *L. nigrinus* must increase by 10 to 20 times in order to meet projected needs. Future laboratory research will focus on understanding the conditions required for adult survival through aestivation; specifically, the effect of photoperiod and temperature during aestivation will be investigated.

Conclusion

Effective field evaluation and improvement of mass-rearing techniques are critical for the success of this biological control program. It appears that *L. nigrinus* has potential to survive in Virginia's climate and is an efficient predator of HWA. Results from this past year indicate some improvement in rearing success, however, mortality rates must be further reduced. Success in rearing is essential for the mass release of *L. nigrinus* in the field. Open field releases will enable further evaluation of *L. nigrinus* as a biological control agent for HWA.

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